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Biology of the Flea Beetle, Altica Carduorum Guer. (Coleoptera: Chrysomelidae) on Canada Thistle, Cirsium Arvense (L.) Scop., in South Dakota

Burton Danis Schaber

BIOLOGY OF THE FLEA BEETLE, *ALTICA CARDUORUM* GUER. (COLEOPTERA: CHRYSOMELIDAE) ON CANADA THISTLE, *CIRSIUM ARVENSE* (L.) SCOP., IN SOUTH DAKOTA

BY

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Abstract

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Under the supervision of

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A laboratory colony of Altica carduorum Guer. was established from a stock colony of 50 adult beetles. They had an average preoviposition period of 7 days when exposed to a regular cycle of 16 hr. of light (24° C) and 8 hr. of darkness (12.75° C). Under these conditions, females laid an average of 259.3 ± 9.7 eggs, and longevity averaged 100 days. The eggs are laid on the underside of leaves along edges of leaf veins throughout June. Adults fed and overwintered in the soil, and some beetles emerged the following spring and laid viable eggs. Laboratory studies indicate that high temperatures and/or low RH are limiting factors to beetle survival. Preliminary findings suggest that the beetle may be reared on southern corn rootworm artificial diet which either contains or does not contain Canada thistle

extract. Results of field studies suggest that this beetle can be colonized in South Dakota. One of the major factors limiting the establishment of field colonies are the predators, Lebia viridis and Harpalus pennsylvanicus. A. carduorum originates from central and south Europe which has a mediterranean type climate where rainfall is heavy, and the humidity is high. Therefore, establishment of A. carduorum in South Dakota, where the summers are usually hot and dry, will be most difficult. Suspected mycoplasma-like bodies were found in sieve elements of Canada thistle plants displaying symptoms of yellows disease.

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In the United States half of the 500 major weeds are introduced species. Thirteen of the top 15 noxious weeds are introduced or are exotic species, therefore, the future for biological control by the classic method, i.e. the importation of exotic phytophagous insects to control introduced weeds offers researchers and scientists a seemingly unlimited future in biological control.

Biological control of weeds may be defined as the study and utilization by man of plant-feeders and/or pathogens for the reduction of host populations below economic levels.

The first successful movement of a species from one country to another for biological control occurred in 1762 when the mynah bird of India was imported to the Island of Mauritius to control the red locust (Moutia and Mamet 1946). McCook (1882) discussed a procedure used by Chinese citrus growers since ancient times, of using a predaceous ant, Oecophylla smaragdina F., in mandarin orange groves to control the numbers of foliage-feeding insects. During the 18th century, predation was the most prevalent form of insect pest control. Kirby and Spence (1815) reported that 6-8 specimens of an hemipteran predator placed in a sealed room thoroughly

infested with bed bugs would completely devour the latter within several weeks.

Biological control concepts of today are based on the phenomenon that one species' population has an immediate and functional relationship to that of another species. This hypothesis appeared as rebuttal to the theory of Malthus (1803) that populations tend to increase in a geometric ratio and their food supplies by an arithmetic ratio. Early in the 19th century Verhulst (1838) formulated population growth curves which showed that populations do not increase indefinitely in a linear fashion, but follow a sigmoid or 'S' curve. Verhulst's work was not recognized until Pearl and Reed (1920) brought it to the attention of the scientific community and showed its significance in population studies. Similarly, other early 19th century observations were reported by Erasmus Darwin, who noted that cabbage caterpillars would increase in numbers but that a certain proportion (about 1/2) would annually be destroyed by a larval parasite (Riley 1931).

Biological control in the United States was stimulated in the 1860's by Asa Fitch (1856) who wrote on the distribution and multiplication of the wheat midge, Sitodiplosis mosellana (Gehin). His writings

influenced Charles V. Riley to distribute parasites of the weevil, Conotrachelus nenuphar (Hbst.) from one locality to another in 1870, thus establishing biological control in the United States.

The first procedure that could be considered as a bona fide biological control method was initiated in 1888 as a direct result of C. V. Riley's address to the Convention of the California Fruit Growers, where he outlined his proposals for a project against the cottony-cushion scale in California. As a result of this address biological control became firmly established in the United States 20 years later.

The striking success of Riley's experiment in biological control stimulated many nations to initiate such projects. Over 200 outstanding successes in biological control have been accomplished in 60 countries, including Australia, Canada, Chile, Fiji, Hawaii and the United States (Doutt & DeBach 1964). However, most of these have dealt with the control of insect pests.

Biological Control of Weeds.- Employment of biological control of weeds has been approached rather hesitantly for 2 reasons: (1) fear that the risks involved are too great compared with the chances of success, and (2) the conflict in general acceptance that a given plant is a

weed, coupled with the fact that introduced natural enemies of weeds would be free to move into other lands where the plant may be considered valuable (Huffaker 1957). Ever since the advent of agriculture, man has engaged in a seemingly endless struggle against weeds. The term 'weed' can be defined as a plant that is found in the wrong place at the wrong time. For example, yellowstar thistle is considered to be a plant pest in the plains states because of the damage to grazing lands, but in California it is beneficial to the bee industry, and fruit and seed crop growers (Huffaker 1957). In Australia the clearing of millions of acres of the prickly pear, Opuntia spp. met with no resistance; however, in Hawaii, vigorous objections were voiced by cattlemen because the tree cactus, Opuntia megacantha Salm-Dyck., was also beneficial as a food and a source of water on some ranges during dry spells (Fullaway 1954).

In the United States, losses incurred from exotic and indigenous weeds are almost equal to the combined losses caused by insects and disease. These losses are second only to those caused by soil erosion (King 1966). According to the United States Department of Agriculture (1965), during the decade of the 60's the annual losses incurred because of weeds was $5.1 billion.

With an ever-increasing outcry by the public against supposed indiscriminate use of herbicides, insecticides, and other pesticides, attention has been focused on the need for an effective, inexpensive and long-lasting control method. Weed control by the use of insects is well documented, and therefore, is no longer a dangerous untested theory when established safeguards are followed.

Any insect that limits its attacks to an individual plant species or a few closely related species may be used as a controlling agent. The most effective agent, therefore, would be one which is very host-specific, such as plant pathogens, nematodes, and certain insects (Van de Laan 1967; Andres and Goeden 1971).

Weed control should not result in plant eradication but in the reduction of the population densities of the weed below an economic threshold. Control, but non-eradication, is important because the reproduction of the specific insect is closely linked to the presence of the weed host plant. Consequently, one wishes that an equilibrium, which lies below the economic threshold, will be reached between the weed's average population density and the population density of the specific insect.

A weed controlling organism may destroy individual plants directly through destruction of vital parts, or indirectly by: (1) creating favorable conditions for infection by primary plant pathogens, or (2) cancelling or disrupting competitive advantages of the weed within the environment (Huffaker 1957). However, the main objective in biological control of weeds and of an ideal controlling agent is not what form of injury does it cause, but rather, does it cause the destruction of 'existing stands' of the weed (Huffaker 1957)?

Zeiger (1967) and Maddox et al., (1971) reported on the control of the introduced alligatorweed, Alternanthera phylloxeroides (Mart.) Griseb., which showed that heavy destruction of foliage and aerial structures of the weed by insects or other agents may not produce desired control unless destruction occurs at the proper time in the plants' life cycle. For example, Zeiger (1967) pointed out that webworms were often found on alligatorweed causing heavy defoliation at a time when its energy reserves were high with little effect on the plants' survival.

The 'synchronized feeding' of adults and larvae of a Chrysolina (Coleoptera: Chrysomelidae) beetle on the basal foliar rosettes of the Klamath weed over a long period in the fall, winter, and spring prohibits

the root from obtaining an adequate supply of nutrients, causing them to 'disintegrate' during the long dry summers in California. Based on this observation, an effective biological control agent derives its energy from its weed host at a time critical to the latter's survival (Holloway and Huffaker 1952).

It is hoped that this introduction to principles of biological control with a brief history will make the following review of some of the major outstanding examples of biological control of weeds more meaningful.

The classic example of biological control of weeds occurs with the prickly pears, Opuntia spp., in Australia. All species of Opuntia found in Australia are of American origin. In 1925, approximately 60 million acres of land were infested, 30 million acres so heavily that the land was essentially useless. Of the 48 species of insects imported for control, only 12 became established (Wilson 1960). The most significant success was obtained with a pyralid moth, Cactoblastis cactorum (Berg) [Lepidoptera: Pyralidae]. Within 5 years of its introduction, millions of acres formerly occupied by Opuntia spp. were returned to useful agriculture (Holloway 1964).

The pasture weed, Opuntia megacantha, introduced

into Hawaii during the 19th century from Mexico, had by 1930 occupied 30,000 acres of a 300,000 acre improved cattle ranch. After overcoming the opposition by owners of unimproved ranches in 1948, the following insects were introduced with the result that control was obtained within 5 years: "the cochineal scale, Dactylpuis opuntiae (Ckll.) [Homoptera], and a cerambycid borer" (Fullaway 1956).

The first successful case of biological control of a native weed by intentionally introduced natural enemies occurred with a project in California directed against 2 prickly pear cacti and their hybrids infesting 62,000 acres of range land on Santa Cruz Island. D. opuntiae, introduced in 1951, reduced the prickly pear cactus by at least 50% by the early 1970's (Andres and Goeden 1971).

In California the Ilamath weed, Hypericum perforatum L., was estimated to occupy in excess of 2 million acres of rangeland in 1944. In 1950 a root borer, Agrilus hyperici Creutzer [Coleoptera: Buprestidae], a cecidomyiid gall fly, Aeuxidiplosis giardi (Kieff) [Diptera: Cecidomyidae], and Chrysolina variana (Scholl) [Coleoptera: Chrysomelidae] were released. By 1956 the Klamath weed had been reduced

from the status of an important pest of range lands to a casual roadside weed (Holloway 1956). Today this weed occupies less than 1% of its former abundance and is no longer included on the list of California's noxious weeds (Huffaker and Kennett 1959). McLeod et al. (1962) reported that in Canada, two attempts at biological control of Klamath weed, and toadflax, Linaria vulgaris Mill., failed.

A current project on biological control in southeastern United States is the first attempt at controlling an aquatic weed. In 1963 alligatorweed had infested approximately 100,000 acres in 8 states from North Carolina to Texas, and California. In 1964 a leaf and stem feeding Agasicles flea beetle was released near Savannah, Georgia (Hawkes et al., 1967). A second release of adults near Jacksonville, Florida, was made the next year. By 1966, adults by the hundreds of thousands were observed at these release sites. Maddox et al., (1971) reported that at a site near Charleston, South Carolina, alligatorweed was reduced 90% from its former level, after the beetles had done a high degree of damage to stems and leaves. This allowed a competitor, aquatic smartweed (Polygonum sp.) to increase, thus helping to choke out the

alligatorweed. In this instance, the feeding of Agasicles had reduced the vigor of the alligatorweed, which lessened its ability to compete with the surrounding plants (control by an indirect method). Due to the effectiveness of this controlling agent, many waterways have since been cleared of alligatorweed.

Biological control of Canada Thistle.- Spurred by the numerous successes in biological control of weeds the Research Institute, Canada Department of Agriculture, and the United States Department of Agriculture, Albany, California, imported from Switzerland in 1964, a flea beetle which attacks Canada thistle. This plant is not native to Canada, as its name suggests, but rather it is indigenous to Europe, western Asia and northern Africa. It is from Europe that the weed was introduced to North America in 1777 (Dewey 1901). By 1926 Canada thistle had spread across the continent from the east coast to the Pacific coast states, south into Kansas, and Missouri, with one isolated patch in Mobile, Alabama (Detmers 1927).

Canada thistle has been known from very early times but was scientifically named by Carl von Linne in 1753 as Serratula arvensis. He described it as having lanceolate, dentate leaves with spinose margins and

having creeping roots. Scopli (1772) placed it in the genus Cirsium where it has remained.

Canada thistle, being a perennial weed, is very difficult to eradicate and is one of the most feared weeds in the United States (Kinch 1967). It has been declared to be 1 of the 8 primary noxious weeds of South Dakota by the South Dakota Weed Control Commission. It has a complex root system, consisting of the fibrous absorbing organs and horizontally creeping, branching roots which serve as organs of storage and vegetative propagation. The latter vary in depth below the soil from a few inches to 3 feet. In crop land they are usually at a greater depth than that reached by ordinary cultivation. The root system continues growing each season thereby spreading out from localized patches. Dissemination of seeds by wind and water currents may disperse the plants to uninfested areas where they can germinate and start new infestations should they find a suitable environment (Detmers 1927).

The types of losses caused by Canada thistle are: (1) crowding out or reducing the growth of the desired crop, causing losses in yield and quality; (2) increased costs due to increased cultivation; (3) increased costs for special seed cleaning; and

(4) depreciation in value of crop land and agriculture of a community. The first of these is probably the most prevalent type of loss incurred by the farmer.

Derscheid and Wrage (1972) reported that "2 Canada thistle plants per square yard reduced wheat yield 18%, while 19 plants per square yard reduced yields 36%." Hodgson (1968) found that "2 shoots per square yard can reduce spring wheat, Triticum aestivium L., yield by 15%, and that 25 shoots per square yard reduced yield by 60%."

Many weeds inhibit seedling growth of other plant species. Retig et al., (1972) showed that if 4 weed seeds are planted next to 1 crop seed of cabbage, (Brassica oleracea L.), and tomato (Lycopersicum esculentum Mill.), abnormal changes in anatomy of cabbage and tomato roots occur, such as inhibited cell elongation, disruption of the epidermis, and disorganization of root tissue. Change in root anatomy could also explain the drastic reduction in yield due to the presence of Canada thistle.

In South Dakota in 1969, Canada thistle infested approximately 230,000 acres on 22,000 farms. A revised report, 3 years later, showed an increase of 133,000 acres of infestation on 2,000 additional farms. If

this acreage is added to South Dakota's 7 other primary noxious weeds, the total acreage infested is approximately 3,000,000 acres. Only bindweed (creeping jenny) infests more (1,500,000) acres than Canada thistle in South Dakota (Derscheid and Wrage 1971).

Studies utilizing Ceutorhynchus litura (F.) [Coleoptera: Curculionidae] and a Cassida spp., [Coleoptera: Chrysomelidae] in the control of Canada thistle are being conducted by Lance Nearman at South Dakota State University. These studies have recently been initiated, therefore, no results are yet available.

It is obvious that cultural and mechanical means in South Dakota are not controlling the rapid spread of Canada thistle. This problem led to the initiation of a biological control project in 1970 involving the release of an introduced plant enemy, a flea beetle, Altica carduorum (Guérin-Méneville, 1858) [Coleoptera: Chrysomelidae], to determine if establishment would occur and the beetle's effectiveness for biological control of this weed. Other facets of this project are being conducted by other researchers at South Dakota State University at the present time.

The initial study of this flea beetle as a possible

control agent was started in June, 1961, at the European Station, Commonwealth Institute of Biological Control, Delemont, Switzerland by M. Karney. His original specimens were obtained from a single locality near Brig, Valais, Switzerland. He studied the biology of the adult and larva and performed some host plant specificity studies, but they were inconclusive (Karney 1963).

Harris (1964) conducted host specificity tests of A. carduorum on 5 tribes of the family Compositae. Fourteen species were from the plant tribe Cynareae. Plants of the 5 tribes were tested and some beetles survived, with sustained feeding on only a few genera within the subtribe Carduinae. He concluded that in nature A. carduorum probably attacks only C. arvense. Zwölfer (1965a, 1969) in an extensive survey of thistle insects in west and central Europe, has never found it on any other plant species. Further laboratory host specificity tests (Zwölfer 1965b) supported the conclusions of Harris and Karney that the host range of A. carduorum comprises the carduinne genera Carduus-Cirsium-Silybum. Zwölfer delineated the geographic range and some ecological factors which may affect the biology of the beetle.

A trial release of 23 beetles at Belleville, Ontario, in 1963, was unsuccessful. Ninety percent of the eggs laid by these beetles disappeared overnight. However, at Lacombe, Alberta, a release was more successful when 1080 beetles were liberated and a small colony survived for three years (Peschken et al., 1970).

Studies on the control of Canada thistle (Zwölfer and Harris 1966, and Zwölfer and Eichhorn 1966) have shown the possibility of using other insects to control this pest weed.

Mycoplasmas.- As a part of this study of biological control of Canada thistle, an investigation was undertaken to determine whether mycoplasma-like organisms are associated with the suspected yellows disease in Canada thistle, and to study the development of these organisms in plants. Hopefully, they can be propagated as another biological control agent of the noxious weed, Canada thistle.

Prior to the discovery by Doi et al. (1967), yellows diseases were thought to have been caused by a virus or virus-like particles (Protsenko 1959), but the pathogenicity of these particles was never demonstrated. Failure to isolate the causitive agent

permitted a new hypothesis, that the causitive organisms were not viruses but rather microorganisms which resemble mycoplasmas. Doi et al. (1967) were the first to suggest that the causitive organism of yellows disease may be a mycoplasma-like or chlamydia-like organism in sieve elements of yellows-infected plants. It is now estimated that this group of disease organisms causes more than 40 plant diseases (Whitcomb and Davis 1970a). This conclusion is based on the evidence of morphological and ultrastructural similarities (Davis and Whitcomb 1971): (1) the bodies are bound by a single unit membrane; (2) the bodies are devoid of a cell wall; (3) the bodies are highly pleomorphic; (4) the bodies are not known to be derived from bacterial parents; and (5) the bodies can be cultured in cell-free media (Hampton et al. 1969). Recently other researchers have demonstrated the association of such organisms with yellows diseases (Dale and Kim 1969; Hampton et al. 1969); Story and Halliwell 1969; Whitcomb and Davis 1970a and 1970b; Maramorosch et al. 1968; Granados et al. 1968; Giannotti et al. 1968; Bowyer et al. 1969).

Kunkel (1924) demonstrated that aster yellows are caused by an infectious agent and transmitted by

leaf-hoppers. Similarly, Sinha and Paliwal 1969; Story and Halliwell 1969; Raine and Forbes 1969; Brcak and Kralik 1969; Giannotti et al. 1968; Granados et al. 1968; Hirumi and Maramorosch 1969; Maramorosch et al. 1968; Shikata and Maramorosch 1967; have located mycoplasma-like organisms in leaf-hoppers. Hampton et al. (1969) established transmission of mycoplasma-like organisms by the pea aphid.

Outline and Objectives.- The general purposes of this research are to investigate and evaluate A. carduorum as a biological control of Canada thistle. The following outlines the objectives:

I. Attempt to establish a laboratory colony.
   A. To study its life history.
   B. To determine the effects of various temperatures and humidity on mortality and fecundity.
   C. To determine preference for ecotypes of Canada thistle.

II. Attempt to establish field colonies by making releases at regions with different climates within South Dakota.

III. Attempt to record factors limiting establishment.

A. Record the number of parasites infecting all life stages.

B. Record the number of possible predators of all life stages.

IV. Attempt to determine the agent causing yellowing.

V. Description of the external morphology of the adult.

MATERIALS AND METHODS

The Host Plant.- The subsequent description of Canada thistle is based on that given in botanical manuals of Fernald (1950), Britton (1913) and Gleason & Cronquist (1963).

The stems are green, sometimes striate having a glabrous surface or with the surface sparsely white tomentose or hirsute (with bristling hairs). The paniculate branched stems are usually from 30 cm to 1.5 m in height.

The leaves are green on both surfaces. The upper surface is glabrous, sometimes shining or with a few short white hairs on the young leaves. The under surface of mature leaves is glabrous, with the young and/or leaves close to the ground being white tomentose beneath. The shape of the leaves are lanceolate to oblanceolate with the acute apex terminating in a spine. The margin of the leaf varies from crenulate (deeply lobed) to ruffled. These acute lobes terminate in a spine with marginal spines or prickles extending to the base of the lobe. The sharp, rigid spines may reach up to 8 mm in length. The base of the leaf is sessile, with the large leaves being decurrent. These decurrent leaf bases

are sometimes mistaken as spines of the stem. The cymose inflorescence has numerous heads which are sessile or peduncled. The involucre is imbricate with a reddish purple, glabrous corrolla. The flowers are fragrant.

Canada thistle can be distinguished from other thistles by its deep green, intensely spiney leaves, small heads of flowers borne in clusters, by growing in patches, and by its horizontal branching roots.

Three distinct varieties (=host-types, ecotypes) were located in the Brookings research areas. The following is a brief description of each of these host types:

a)--horridum. All of the leaves are crenate (ruffled) pinnately lobed. These lobes are very narrow and strongly spinose. This variety was found on the Kieckhefer, Durland and Spearfish release sites, and will, hereafter, be referred to as host-type 1.

b)--mite. The stem leaves are sinuate-pinnatifid, subundulate (usually not crenate), whereas the branch leaves are subentire or denate, and minutely spinose. This variety was found on the Johnson release site, and will, hereafter, be referred to as host-type 3.

Separated from the Johnson release site by a hedge was a variety having characteristics of both

of the previously described host-types. This host-type was used during the host-type preference tests and is, hereafter, referred to as host-type 2.

Characteristics used in separation of the above host-types are some of those used by Hodgson (1963) in his description of 10 ecotypes of Canada thistle collected from locations in Montana, Idaho, Washington, and Wyoming.

Collection of underground shoots of host-type 3 were dug each fall and placed in 3 gallon plastic bags, with just enough soil to cover the shoots. These bags were placed in a warm place for 1 week enabling the roots to sprout, then placed in a large walk-in cooler kept at 1-2° C. During the winter months, 5 shoots per pot were planted in 15 cm plastic pots at regular intervals, then placed in the Northern Grain Insect Research Laboratory greenhouses. The plants were grown at a temperature of 24° C, with 16 hours of light. Plants were watered only twice a week. These plants were carefully watched because of a mite, Oligonychus partensis (Banks) [Tetranychidae: Acarina] which can easily wipe out the entire greenhouse stock in a very short time.

The Control Agent.- The original description of

the adult beetle by Guérin-Méneville (1858) and a later description by Karney (1963) have been superficial, therefore, I will give a complete morphological description in a later section of this paper.

Laboratory Rearing.- A laboratory colony of A. carduorum was established at South Dakota State University from a stock of 50 adult beetles, shipped from the United States Department of Agriculture, Biological Control of Weeds Laboratory, Albany, California.

The technique used for the rearing of these beetles is as follows: Twenty-five to 50 adult beetles were placed in one gallon cardboard ice cream containers, of which the central portion of the lid was removed. A piece of plastic wrap was placed over the container and the lid slipped into place, then holes were pierced in the plastic to allow for gas exchange and to prevent condensation. A bouquet of cut Canada thistle was made by inserting thistle foliage into a 6-dram snap-cap vial, filled with water. The plant material is held upright by wrapping it with cotton and inserting it through a hole in the cap. Each day the bouquet was removed and replaced by a fresh one. The old bouquet was thrown away, or if eggs were present

it was placed in a separate container until ecolsion occurred. If the bouquet was left for 2 or 3 days in the adult cages, too much leaf area was eaten and the plants desiccated, in turn causing the eggs to dry and shrivel. The original bouquets taken from the adult cages were replenished with water and placed in 1/2 gallon plastic ice-cream containers. These containers were covered by cellophane held fast by the lid rim. Once again holes were pierced in the plastic. After the eggs had hatched a fresh bouquet was added to the same container with as much contact as possible with the older bouquet in order that the larvae could transfer to new foliage. After 3 days, or when the larvae were in the 2nd instar, a 1/4 inch layer of foam plastic was placed on the floor of the container to absorb condensation, thus preventing the drowning of many larvae. During the summer months fresh bouquets were collected from the field each day. During the winter, stock was obtained from a greenhouse supply of approximately 400 potted plants. When a few mature larvae were found on the floor of the container, the bouquet was transferred into 30 mm clay pots filled 3/4 full with a sterilized mixture of 1/3 black dirt, 1/3 sand, 1/3 vermiculite. A plastic cylinder 30 mm

in diameter and 30 mm high was placed in the pupation chamber. This allowed 3rd instars falling from heavily laden bouquets to burrow immediately into the soil to construct their pupation cells. Reduced adult emergence occurred if the larvae were allowed to drop to the floor of the larval growth chambers. Similarly, if the young larvae were moved or touched with a camel hair brush, many failed to develop further. The soil had been thoroughly moistened before putting in the thistle bouquets. Every other day 400 ml of water were placed in the bottom saucer under the clay pots. This allowed the soil to absorb moisture and reach an equilibrium, thus enabling the larvae to seek their own moisture level when pupating. Adults started to emerge from the soil in about 2 weeks, thus beginning a new cycle. This procedure has been adapted from the procedure used at Albany, California (Hawkes, personal communication).

The growth chamber was kept at a daytime temperature of 26.6° C and a night temperature of 18.3° C, 16-hour day length, with a RH above 50%.

Laboratory Studies.- Fecundity.- Three ecotypes of Canada thistle and one of bull thistle, Cirsium vulgare (L.), (host-type 4) were used in a fecundity

experiment.

Field studies showed that there are at least 3 ecotypes (host-types 1-3) of Canada thistle in Brookings County. Since a knowledge of host preferences would help in obtaining in the laboratory a maximum number of eggs and a greater hatch, the study of fecundity of A. carduorum was conducted. Three replications of the 4 host-types were run. Each replicate had one large leaf placed in a 6-dram snap-cap vial. Each day the leaf was removed and replaced with a fresh one. The eggs laid on the leaf were counted, the number was recorded, and the eggs were placed in a soda fountain cup, 15 mm high. The leaf in the soda fountain cup was observed every day for 15 days, and the number of larvae hatching each day was recorded. If the eggs had not hatched within 15 days they were considered to be infertile and were discarded. (It had been previously determined that no eggs hatched after 15 days.) As soon as all the eggs had hatched these 1st instar larvae were transferred to rearing chambers containing the other larvae from the same replicate. Fully grown larvae were transferred to pupation chambers.

Adults emerging on July 13, 1971, were paired and placed on the 4 host-types of thistle.

The above experiment was conducted under the following environmental chamber parameters: temperature 24° C day, 15° C night; 50-70% RH, day-length 16 hours.

The hatchability of the eggs of the 4 host-types was compared using an independent chi-square analysis.

Host Preference.- Feeding preference of A. carduorum was determined by placing 4 pairs of adults into 4 2-gallon battery jars each containing bouquets of the 4 host-types.

Every 1/2 hour the number of each sex was recorded per host-type, starting at 8 a.m. and terminating each day at 4 p.m.

Only the observations taken on the 2nd and 3rd days were used in the analysis of these data because randomization had not yet been complete on the 1st day, and by day 4, some leaves had deteriorated, thus shifting beetles to secondary host preferences.

Feeding Unit Analysis.- The second phase of this experiment involved the determining of the number of "feeding units" per host-type. A "feeding unit" had been defined as an area equivalent to the cross section of a wax match (1.5-2.0 mm^2)(Zwölfer 1965a, Frick 1970). I determined a "feeding unit" using a dissecting microscope, equipped with an optic reticule. At 12X,

1 square equals 1.67 mm^2. Therefore, for ease in computation, 1.67 mm^2 was chosen as a feeding unit in this experiment.

Longevity Study.- To determine longevity under laboratory conditions, 3 containers, each with 28 newly emerged beetles (21♀ - 7♂) were observed every day until all members of the experiment had perished. As in the other experiments, fresh bouquets of host-type 3 were placed in the chambers as needed. Each day the dead beetles were removed into separate vials with their number and sex recorded.

Temperature and RH Study.- Studies to determine the effects of different temperatures and humidities on fecundity were carried out during the summer of 1971. A secondary purpose was to correlate, if possible, these laboratory data with the data recorded in field experiments, thereby enabling one to predict population trends under similar conditions in the field. Test parameters were a) 24° C day, 15° C night, 50-70% RH; b) 30° C day, 21° C night, 70-100% RH; c) 30° C day, 21° C night, RH less than 40%; d) 35° C day, 21° C night, 40-60% RH; e) 35° C day, 21° C night, 60-80% RH.

Each experiment consisted of 3 replications, each containing 1 male and 1 female with the environmental

chamber kept at one of the above set of parameters throughout the experiment. Each day, as in the host-type preference studies, the bouquet consisted of 1 large leaf. Each bouquet was changed daily and the number of eggs laid was recorded. Subsequently, the number of eggs which had hatched were recorded. By comparing the emergence in each experiment, the best environmental parameters for the rearing of laboratory adults were obtained. These data were analyzed by the use of an independent chi-square analysis.

Artificial Diet Study.- Artificial diet studies were conducted by using newly hatched larvae obtained from thistle bouquets. A wet camel hair brush was used to remove larvae which were less than 24 hours old and to place them on 10 ml of artificial diet in a 1 oz plastic cup. The diet was prepared and formulated according to the method described by Sutter et al., (1971). In addition, an aqueous extract from Canada thistle was added. The extract was prepared as follows: a one gal bag of frozen Canada thistle leaves were added to 1800 ml of water in a Waring blender and blended for 3 minutes at high speeds till the mixture was fully homogenized. This mixture was filtered through 4 layers of cheesecloth using a Buchner funnel. The solute was

then centrifuged at 100,000 rpm for 30 minutes, after which it was once more filtered through a Buchner filter, poured into 50 ml bottles, frozen and stored. When a batch of diet was made this solute was thawed and run through a millipore filter. Next, 50 ml of this solution were added to a batch of diet making 50 1 oz. cups, or approximately 500 ml of diet.

In a preliminary experiment, unaltered corn root-worm diet was used. One hundred and twenty-five larvae - less than 24 hours of age - were placed, 5 to a 1 oz. cup, and allowed to develop. The adults obtained from this study were then paired.

In another study, 3-5 larvae were placed in each cup, the cups inserted into trays of 25 each, and the trays then placed in a growth chamber with the following parameters: temperature 28° C, 75-80% RH, with 16 hours of day light.

Rearing beetle larvae on successful synthetic diets could simplify procurement of winter food supplies. Such a technique would reduce by many man hours the work needed in collecting, cutting, and arranging thistle bouquets.

Field Studies.- The study areas consisted of 2 stations in South Dakota. The eastern station was

located on the Coteau des Prairies, a region having Chernozen type of soil. This region is a highland located between the Minnesota-Red River Lowland on the E, and the James River Lowland on the W. Elevation here ranges from 1600 ft above sea level to 2000 ft (Westin et al., 1967). This station contained 3 release sites, viz. one at the community of Sunnyview, 2 miles N of Brookings, on gently sloping to medium-textured soils of the Vienna Lismore series in a semi-sheltered area at an elevation of 1625 ft.

A second eastern site was situated in Moody County on the Durland farm (1 mile S of the Big Sioux River on County road #21), on Lamoure silt loam soil. This site had 2 release areas, one very sheltered, the other in open prairie about 200 yards away. Elevation at these 2 release areas was approximately 1580 ft.

A third eastern site was situated on the Johnson farm, Brookings Co., approximately 4 1/4 miles north and 1 1/2 miles west of Brookings on Estelline silt loam, nearly level-medium to fine-textured soils in a well sheltered area at an elevation of 1646 ft above sea level.

The other research station was located approximately 3 miles N and 3/4 miles W of Spearfish on

US 81, on the banks of Spearfish Creek. Elevation at the research site is about 3000 ft (Westin et al. 1967) and the soils are in the Lohmetler-Glenberg-Harrison series.

Each of these sites were selected after intensively scouting the area and determining that they contained an easily accessible, large and nearly pure stand of Canada thistle.

Field cages 2' x 2' x 4' were constructed using 1" x 2" wood strips. They were built to allow entry by 2 openings. Besides the front door there was a second opening, the 6" top section which could be flipped back. The top of the cage consisted of a sheet of 1/4" plexiglass which allowed unobstructed observation. The top section was opened for observing the adult beetles, because they fall earthward when disturbed. Thereby the likelihood of losing them through escape through the front door was lessened. However, when eggs and larvae were present, it was much easier to observe them by using the front door.

A hygrothermograph was maintained at the Johnson farm for the duration of the research.

Eight pitfall traps made from 1/2 pint jars were placed on the periphery of the first release site on

the Johnson farm. These were sampled weekly and their catches placed on alcohol.

The purposes of these studies were to determine the response of A. carduorum to climate in South Dakota and to see whether establishment of field colonies could be obtained.

Releases.- The first release was made July 3, 1970, at the Sunnyview site. Fifty larvae were placed in each of 2 field cages, and 100 larvae were released, uncaged, 50 on each of 2 1 m^2 plots. Each subsequent day the number of larvae observed was recorded. In the spring of 1971 observations were made daily for the month of June, and weekly throughout the rest of the summer.

On July 11, 1970, on the Durland farm, 50 larvae were released in 2 cages, and 50 in 2 uncaged release areas. Daily observations were maintained with the number of larvae seen being recorded. The following spring, observations were continued daily until July; then weekly, throughout the remainder of the summer.

A release was made at the Johnson farm site on September 16, 1970, when 485 adult beetles were placed into a cage. This population was followed consistently with daily observations until late August, 1972, when the last of this population had pupated. To supplement

the F_2 generation adults which emerged from the 1970 release, 25 adults from the laboratory colony were added to the same cage on August 3, 1971. During the summer of 1972 the life stages were analyzed using the methods developed by Moyle and Franklin (1955).

On June 4, 1971, a cage was erected approximately 50 ft S of the previous release site to house a new release of 50 adult beetles.

A cage was erected along the W bank of Spearfish Creek at the Spearfish research station on June 7, 1971, and 50 adult beetles were released in it. This site was observed on July 8, 1971, at 6 p.m. and the data recorded. On July 27, 1971, 100 more beetles were released (50 caged, 50 uncaged) at the same location. This site was observed on August 16, 1971, and on June 4, 1972, and the results recorded. A hygrothermograph was maintained at this site by Dr. Burruss McDaniel throughout the summer months of 1971.

Predator Study.- A predator study was conducted at the Johnson farm for which a radioisotope tracer was used to identify the predators of radioactively marked Altica beetles. A 99% pure stock solution (0.30 ml) of P^{32} in the chemical form of H_3PO_4 in 0.02 N HCL, with a concentration of 33.1 mCu per ml was diluted

by pipeting 3 ml of distilled water into the vial containing the P^{32}. Two large Canada thistle plants, about 45 cm high and which had not yet bloomed, were enclosed on all sides by a square of 1 m long pitfall traps. The P^{32} was introduced into the hollow stem near the base of the plants by making a small vertical cut with a knife, and then placing 0.3 cc (containing approximately 2,000,000 cps) of the diluted stock solution into this hollow with the aid of a long-needled hypodermic syringe (Pendleton and Grundmann 1954).

Samples of leaf tissue taken 24 hours later showed incorporation of the radioactivity throughout the plant. After this period, 10 adults and 25 3rd instars were placed on the "labelled" plants, and allowed to feed.

Radioactivity counts were made of the collected fauna using a Mark 13, Model 1, Serial A-174, RCL Sealer for the laboratory analysis. Field detection was done with a portable Geiger-Mueller survey meter.

Samples of insect fauna and plant flora were collected from outside the research area for the determination of background counts.

Mycoplasma Study.- In all 3 summers (1970-72) of the study, diseased Canada thistle plants displaying the classic symptoms of suspected yellows disease were

observed at the Johnson farm site. Specimens were collected and samples of secondary vein material from leaves displaying suspected yellows disease were cut into 0.5 mm x 1.5 cm strips and fixed for 19 hours in 3% glutaraldehyde, then put through a series of buffer rinses, post-fixed for 4 hours in 2% OxO_4 (both in 0.1M phosphate buffer, pH 7). All samples were subjected to a graded acetone dehydration series (25%, 50%, 75%, 100%) for 1/2 hour each, then placed in a thoroughly mixed 1:1 acetone Bojax mixture (Epon and Araldite 502), and allowed to polymerize for 1 1/2 hours. After this, the plant tissue was embedded in freshly prepared pure Bojax mixture and placed in an oven for 48 hours at 60° F. Sections were cut on a Porter-Blum MT2-B ultramicrotome equipped with glass knives and stained with 2% aqueous uranyl acetate for 1 1/2 hours followed by 20 minutes with lead citrate (Reynolds 1963). A RCA EMU-3G electron microscope was used to examine the section. Samples from 3 diseased and 3 healthy plants were sectioned and examined.

RESULTS AND DISCUSSION

Laboratory Studies.- It was found that the 3rd instars pupated within the upper 2 cm of soil. The adult laboratory reared beetles upon emerging displayed marked activity within the rearing chambers which would last for approximately 12 hours. This increased activity was in the form of very erratic jumping around the cage. Each spring I noticed this behavior displayed by the emerging overwintering adults at the Johnson farm release site. This type of activity was first reported by Karney (1963) during preliminary research using A. carduorum in feeding tests.

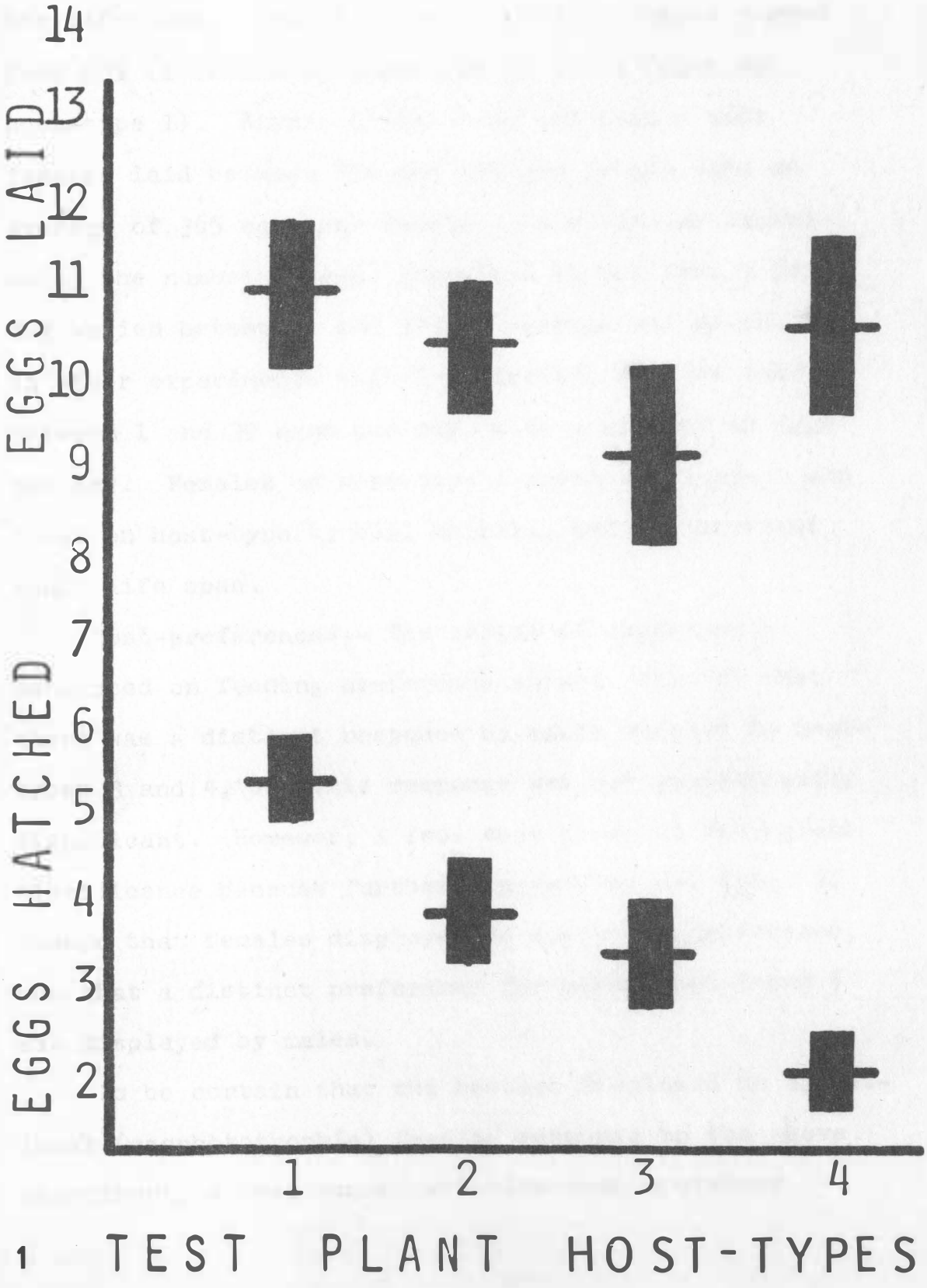
Fecundity.- The total number of eggs laid and hatched, the percent hatch, the total number of adults emerged are shown in Table 1 and Fig. 1. The data were analyzed using an independent chi-square analysis. Host-type 1 was significantly ($P > 0.01$) preferred over host-types 2 and 3. It is suspected that host-types 2 and 3 are significantly different from host-type 4 but the independent chi-square analysis did not allow that comparison. If the total number of eggs laid by all the females are added together the results show that each female laid approximately 315 eggs during

Table 1.- Fecundity and viability of A. carduorum when reared on 4 different host-types.

Host-types	Total No. Eggs	Total No. Hatch	Percent Hatch
1	1137	410[a*]	32
2	1076	257[b]	23
3	778	209[b]	26
4	749	89	11

*Differences are significant at the 99% level when compared values have no letters in common.

Fig. 1.- Mean number of eggs of A. carduorum laid and hatched per host-type.

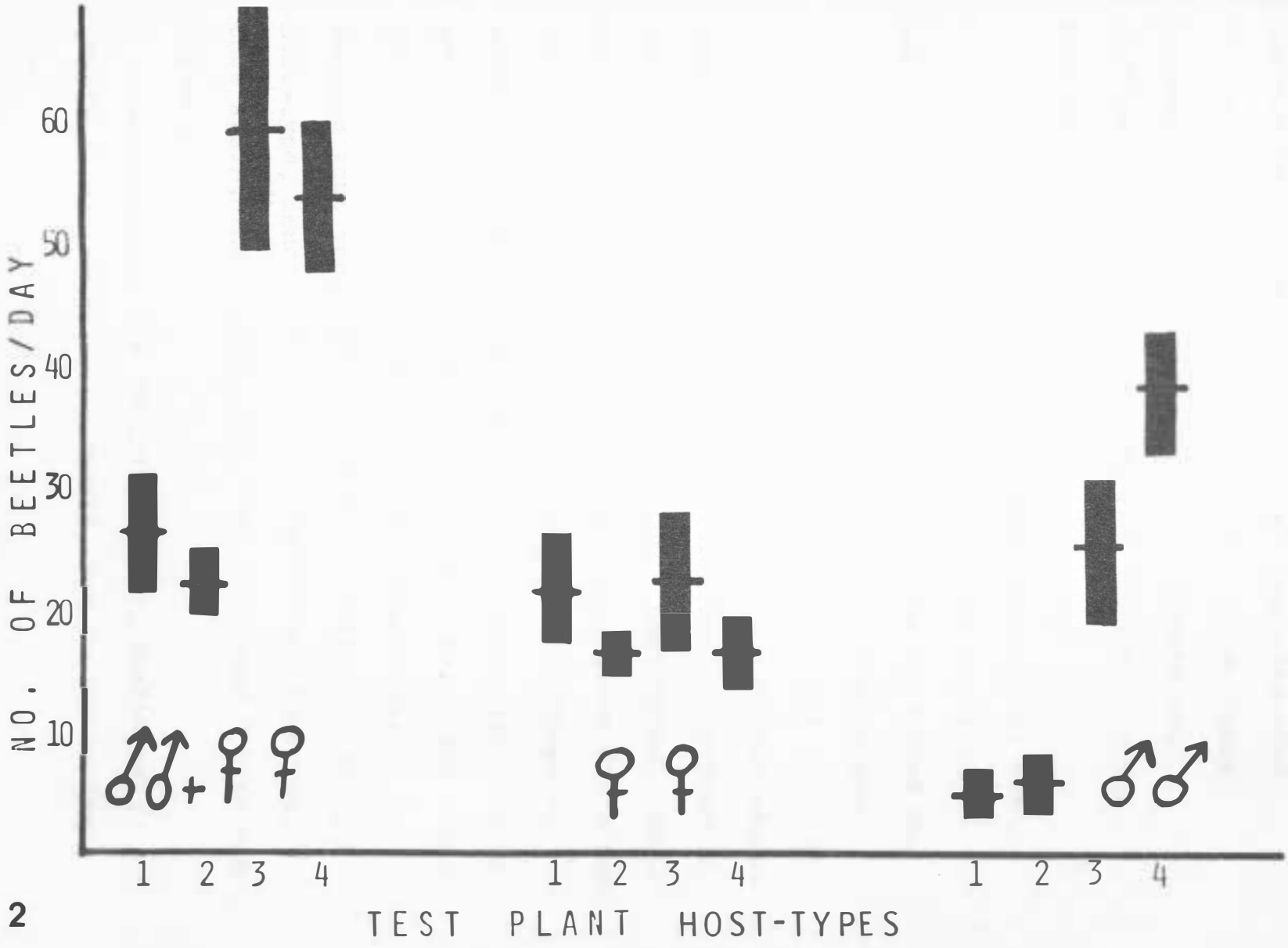

EGGS LAID
EGGS HATCHED
14
13
12
11
10
9
8
7
6
5
4
3
2
1
2
3
4
1 TEST PLANT HOST-TYPES

her life time. Number of eggs laid per female varied from 609 (1 female on host-type 1) to 44 (also on host-type 1). Karney (1963) reported that 6 test females laid between 210 and 491 per female with an average of 365 eggs per female. In a similar experiment, the number of eggs deposited by one female per day varied between 1 and 39; no average was given. In other experiments that I conducted, females laid between 1 and 39 eggs per day, with a mean of 10 eggs per day. Females on host-type 1 lived the longest and those on host-type 4, bull thistle, had the shortest adult life span.

Host-preferences.- The series of experiments conducted on feeding preference showed (Fig. 2) that there was a distinct response by adult beetles to host-types 3 and 4, but this response was not statistically significant. However, I feel that there is biological significance because further analysis by sex (Fig. 2) showed that females displayed no host-type preference, but that a distinct preference for host-types 3 and 4 was displayed by males.

To be certain that the beetles displayed no directional (geophototrophic) feeding response in the above experiment, a test experiment with each container

Fig. 2.- Mean numbers of A. carduorum observed per host-type per day; from left to right - sexes combined, females alone, males alone.


NO. OF BEETLES/DAY
60
50
40
30
20
10
1 2 3 4
1 2 3 4
1 2 3 4
TEST PLANT HOST-TYPES

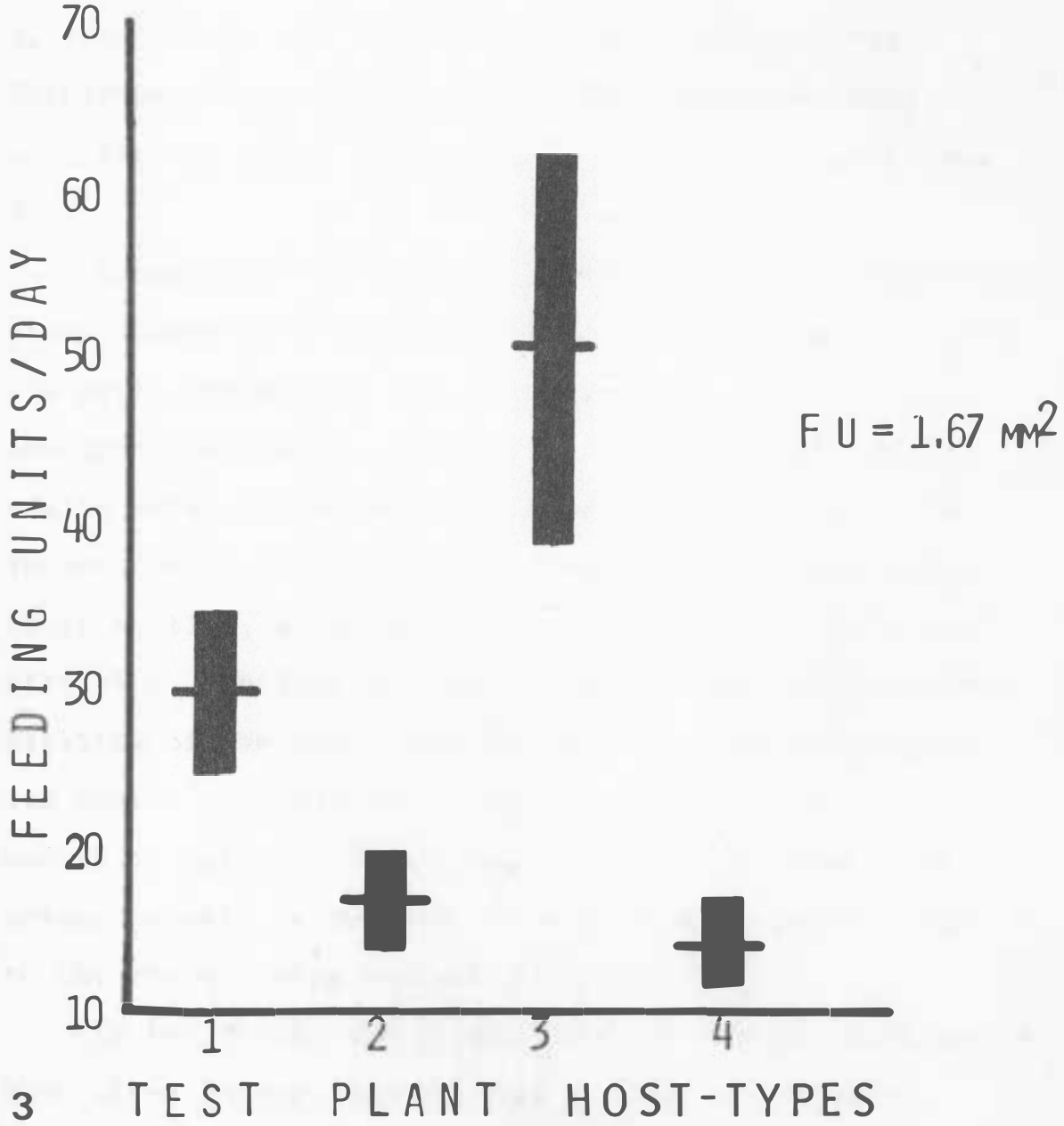
2

containing 4 leaves of 1 host-type was conducted. The results, analyzed by an analysis of variance (one-way classification) showed no significant differences in directional feeding behavior of the beetles.

Feeding unit analysis.- After the beetles had fed on the leaves for the 4 days in the previous experiment, a feeding unit analysis was conducted and the area consumed was measured and the results are shown in Fig. 3. Once again it is noted that although there is no statistical difference among the host-types there can be noted a distinct biological difference in the response of the beetles to the 4 host-types. However, if more replications of this experiment would have been run, it is quite possible that the differences noted could have been statistically indicated. Leaves of host-type 4, bull thistle, are very thick and spongy and contain numerous stiff hairs. Therefore, it is assumed that there is more food per unit area in this host-type, hence, less feeding units were observed. Once again, the beetles preferred host-type 3 over the other host-types.

To summarize the fecundity study, host-type 1 (Table 1) seemed to be preferred, but in the analysis

Fig. 3.- Mean number of feeding units per host-type after being fed upon for 4 days by A. carduorum adults


70
60
50
40
30
20
10
FEEDING UNITS/DAY
F U = 1.67 mm2
1
2
3
4
TEST PLANT HOST-TYPES

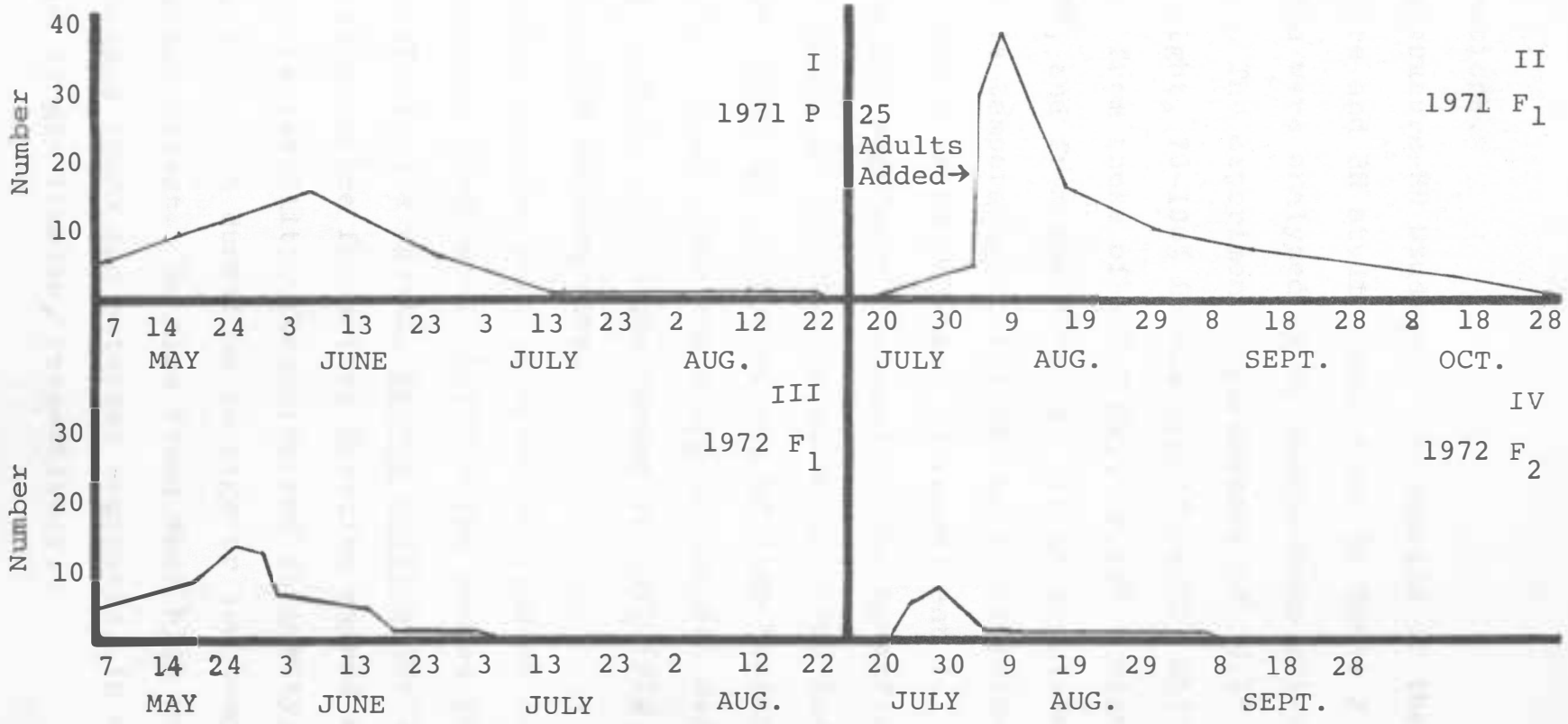
3

by feeding units (Fig. 3) host-type 3 was distinctly preferred. In feeding preference experiments (Fig. 2), the males displayed distinct preferences for host-type 3, compared to the females which were nonselective. Therefore, higher feeding units were observed since more time is spent in feeding by the males on host-type 3.

Longevity.- The results of the laboratory longevity study showed that the oldest individual, a female, lived 159 days, whereas the entire population of the colony averaged 100.0 days. Therefore, in all other tests, adults were considered to be dead after 100 days. The importance of this rather long life span is that these older beetles, although they may not produce any more offspring, continue to feed on leaves, thus reducing the vitality of the plant, and ultimately, contributing to its death. Depredation of the host occurs because the amount of nutrient flow going into seed production of primary growth is reduced, as well as the nutrient flow to the roots during secondary growth.

In the field, the oldest possible beetle could not have lived longer than 105 days. (Fig. 4) However, most overwintering beetles were dead by the time the F_1 adults emerged and, therefore, there is little overlap

Fig. 4.- Number of A. carduorum adults at the Johnson farm release site for the years 1971 and 1972. I. parent generation 1971; II. F_1 generation 1971; III. F_1 generation 1972; IV. F_2 generation 1972.


Number
40
30
20
10
I
1971 P
25
Adults
Added→
II
1971 F1
7 14 24 3 13 23 3 13 23 2 12 22 20 30 9 19 29 8 18 28 8 18 28
MAY JUNE JULY AUG. JULY AUG. SEPT. OCT.
III
1972 F1
IV
1972 F2
Number
30
20
10
7 14 24 3 13 23 3 13 23 2 12 22 20 30 9 19 29 8 18 28
MAY JUNE JULY AUG. JULY AUG. SEPT.

4

of generations.

Temperature-RH Studies.- The results of the temperature and RH studies are shown in Table 2. These data were analyzed by an independent chi-square analysis. The experimental parameters of 29.4° C day, 21.1° C night, 70-100% RH was significantly different ($P > 0.01$) from those of 24° C day, 12.75° C night, 50-70% RH, and from the other 3. It is concluded that not only is temperature critical, as in experiments 3 and 4, but also RH as shown in experiment 1.

Very few eggs were produced at the Spearfish research site, an observation which confirms the laboratory findings of the effect of high temperature and low RH. Also, note the sharp population decline of the F_1 generation in the summer of 1971 (Fig. 4) at the Johnson release site.

Similar results were observed in studies conducted by Le Cato and Pienkowski (1972) which showed that exposure of alfalfa weevil, Hypera postica (Gyllendal) to high temperature for a long duration caused a great reduction in fertility, fecundity and longevity, while exposure for short durations to high or low temperatures had a lesser effect. He also found that high temperatures damaged sperm and retarded oogenesis, in unmated males and virgin females, respectively.

Table 2.- Fecundity and viability of A. carduorum when reared in different laboratory environments.

Experiment No.	Parameters RH	Temp	C	M	Eggs Laid SD	M	Eggs Hatched	SD
1	40%	D 29.4 N	21.1	245.0	212.3	0.3	a*	0.6
2	70-100%	D 29.4 N	21.1	288.6	256.6	134.0	b	117.0
3	50%	D 35 N	21.1	164.0	32.5	1.0	a	1.7
4	60-90%	D 35 N	21.1	259.6	71.2	5.3	a	5.0
5	50-70%	D 23.9 N	12.75	259.3	91.7	69.6	c	61.1

*Differences are significant at the 99% level when compared values have no letters in common.

Artificial Diet Study.- It had been anticipated that 3 different types of artificial diet would be used in this experiment, a)-standard corn root-worm diet, b)-aqueous extract of thistle, c)-powdered form of freeze dried plants. However, due to the low numbers of larvae available only 1 replication of the first 2 were run. The results recorded here are only preliminary, but they do indicate that the rearing of this beetle on artificial diet is possible for some of its life stages.

The results of the diet containing an aqueous extract of Canada thistle are given in Table 3. Larvae under 12 hours of age survived the best. Very good survival rates were noted for the 1st 12 to 15 days at which time the majority pupated. If larvae had not pupated within 15 days, they usually did not pupate at all beyond this time. It is felt that failure of more beetles to emerge as adults was partly due to the fact that a 3:1 vermiculite soil mixture was used in the pupation chambers. Such a mixture did not readily allow the construction of pupal cells. Another factor possibly contributing to the limited success was very high RH within the growth chamber. Temperatures were usually higher than 26° C and many times the RH was at saturation for many days in succession.

Table 3.- Viability of 1st instars of A. carduorum when fed on corn root-worm diet containing an aqueous extract of Canada thistle.

Age in Hours	No. Placed	No. Alive After 12 Days	Percent Survival	No. Pupating	Percent Pupating of No. Placed	Percent Pupating of 12 Days Survivorship
24	75	48	64	10	13.7	21.0
12	75	61	81.5	44	58.5	72
12	141	81	57.5	31	22.0	38.2
4	30	19	63.5	15	50.0	79.0

The experiment using the corn root-worm diet was more successful. Twenty-eight mature larvae were placed in a pupation chamber after 22 days on the artificial diet. Five males and 2 females emerged and 1 pair was placed in a separate rearing container. The female laid 452 eggs over a 64 day period extending from March 16 to June 19, 1971. No eggs hatched, however.

Another pairing of 1 male from the diet and a female from the laboratory colony resulted in the oviposition of 310 eggs, but once again, none of the eggs hatched. However, when 1 male from the artificial diet was placed with a female from the laboratory colony, 425 eggs were laid, and 85 hatched and were placed on Canada thistle bouquets to complete development. Six females and 1 male later emerged.

The significance of the above experiments is twofold: a)-these beetles can be reared on artificial diet (larval stages) and b)-males are fertile and females produce eggs.

Field Studies.- Releases.- At the Sunnyview site, where releases of only larvae were made, adults could only be found which had emerged in cages. Two males and 2 females and 1 beetle of undetermined sex emerged

on July 22 and 23; they were last observed on August 4, 1970. In the spring of 1971 (May), 1 male and 1 female were observed for 3 days. No field establishment of the beetles occurred at this release site.

At the Durland farm, all larvae released disappeared within 3 days. No emerged adults were observed. This site was abandoned during the winter because horses had trampled the release area and had broken the cages to pieces. Possibly, the poor results observed at this site were due to the fact that many of the larvae released were only in the 2nd instar. The weather during the release was very hot and dry and could also have been a contributing factor.

Because of such poor results from larval releases, all subsequent releases were made using adults.

At the Johnson farm, 15 females and 1 male emerged from May 10 to June 5, 1971, (Fig. 4) from the release made during the fall of 1970. This parent generation produced 38 females and 12 males during the summer from August 4 to September 14, 1971. On August 2, 25 laboratory reared beetles were added to the cage of the 50 field reared beetles (75 total). However, on September 14, 1971, (Fig. 4) only 8 beetles were seen. A major population crash occurred between August 14 to

28, when at the same time very high temperatures (Table 4) and low RH (Table 5) were also observed. Similar concurrent population declines were observed at the Spearfish release station.

Fourteen beetles (13 females and 1 male) emerged the following spring (1972) (Fig. 4), even though the maximum number of beetles observed at the Johnson farm after August 29, 1971, was only 10. This indicates either that some adults entered the overwintering state in very early fall, or that some larvae or pupae may overwinter and emerge the following spring as adults. The 14 beetles which emerged in the spring of 1972 produced approximately 1350 eggs, 425 first-, 290 second-, and 225 third instar larvae, from which only 7 females and 1 male emerged during the summer. The last beetle was observed on September 6, 1972. A comparison of the abundance of adults for each year (1971, 1972) is shown in the above mentioned figure. These results are significant because no other investigations in North America have obtained viable eggs from overwintering females, except for a caged release of 1082 beetles at Lacombe, Alberta, Canada.

The 1972 parent generation at the Johnson farm

Table 4.- Comparisons of weekly daytime (high and low) temperatures and nighttime (high and low) temperatures for the summer months in 1971 and 1972 at the Johnson farm release site.

	Temperature											
	Day						Night					
	High		Low		Average		High		Low		Average	
Date	1971	1972	1971	1972	1971	1972	1971	1972	1971	1972	1971	1972
May												
2-8	77	69	58	48	72	60	51	41	28	32	37	37
9-15	89	69	58	54	74	62	71	50	24	42	41	45
16-22	84	91	52	65	70	69	57	60	43	45	49	56
23-29	89	80	46	62	68	72	61	62	32	51	44	56
June												
30-5	83	78	51	58	73	69	62	58	47	42	54	51
6-12	85	83	68	66	77	76	61	66	55	45	58	57
13-19	89	86	85	65	87	74	66	66	60	48	63	55
20-26	92	76	79	62	84	69	68	62	55	43	60	50
July												
27-3	94	86	68	75	79	67	64	60	54	53	63	57
4-10	84	78	72	65	78	73	69	60	52	44	60	53
11-17	85	90	73	65	79	78	67	68	48	54	55	60
18-24	85	82	70	66	77	77	68	68	45	55	52	60
25-31	83	82	69	68	78	76	60	60	42	56	52	58
August												
1-7	85	87	73	66	78	74	53	67	37	45	44	55
8-14	94	85	82	62	87	73	64	60	45	40	58	52
15-21	100	91	83	86	92	89	68	76	56	64	61	69
22-28	104	89	76	58	91	69	67	64	40	52	53	57
September												
29-4	95	84	82	62	88	74	67	65	58	41	63	53
5-11	95	74	65	62	77	69	55	62	41	42	50	50
12-18	72	87	61	62	67	72	37	56	31	43	33	50
19-25	74	79	44	61	64	71	54	54	34	42	43	47

Table 5.- Comparisons of weekly daytime (high and low) RH and rainfall for the summer months in 1971 and 1972 at the Johnson farm release site.

Date	RH Day High 1971	RH Day High 1972	RH Day Low 1971	RH Day Low 1972	RH Day Average 1971	RH Day Average 1972	Rainfall Inches per week 1971	Rainfall Inches per week 1972
May								
2-8	40	84	28	38	34	66	0	2.61
9-15	47	100	28	42	34	67	.03	1.06
16-22	92	64	27	35	46	55	.33	0
23-29	100	100	35	60	64	73	.43	4.78
June								
30-5	100	80	52	58	59	67	1.16	.14
6-12	100	94	65	44	80	68	2.28	.06
13-19	80	100	60	50	67	67	.51	.13
20-26	75	100	56	40	64	65	.30	1.80
July								
27-3	100	68	44	38	71	52	1.25	.42
4-10	88	80	58	42	73	62	.72	1.04
11-17	95	100	48	62	61	70	0	1.08
18-24	100	100	52	58	66	81	.17	2.17
25-31	68	95	54	52	58	70	.24	1.46
August								
1-7							0	.07
8-14	64		48		52		0	.31
15-21	60	64	36	55	47	59	.87	0
22-28	50	100	28	52	35	73	0	1.36
September								
29-4	80	64	40	44	60	56	2.25	.03
5-11		95		50		60	.25	.37
12-18	64	100	39	50	46	66	0	1.33
19-25	100	50	34	40	59	44	.41	.20

produced only 8 F_1 adults. The abnormal high RH during June (Table 5) may have caused the decline in progeny. It was found in the initial stages of laboratory research that if the pupation chambers were watered from above, very poor emergence was noted. However, when the pupation chamber was watered from beneath via a saucer, emergence rates greatly improved. It is possible that the rains in early July, 1972, drowned many of the field larvae and pupae. A comparison of the temperature and RH (Tables 4 and 5) for the 2 years points this out.

At the Spearfish site the temperature (Table 6) was very high during the summer of 1971 and consequently very few eggs were found within the caged release. On June 7, 1971, 50 beetles were released and by July 8 only 8 beetles were observed. On July 27 no adults were observed, but 15 very dried and shriveled eggs were observed.

A second release on July 27, 1971, of 50 caged, and 50 uncaged had similar results as those mentioned above. Observations at this site were made on August 16 when only 6 adults were located within the cage, and none

Table 6.- Weekly daytime (high and low) temperatures, nighttime (high and low) temperatures, RH, and rainfall for the summer months in 1971 at the Spearfish release site.

Date	Temperatures						RH			Rainfall
	Day			Night			Day			
	High	Low	Average	High	Low	Average	High	Low	Average	Inches per week
July										
20-24	113	78	101	64	51	56	60	10	30	0.23
25-31	97	68	83	44	43	43	60	30	45	0.14
August										
1-7	108	62	80	50	39	44	55	12	36	0
8-14	120	100	112	56	46	53	24	10	17	0.14
15-21	102	87	96	65	52	57	26	16	20	0.41
22-29	107	104	104	66	56	61	22	10	18	0

in the field release area. This site was observed again on June 4, 1972, and no eggs, larvae or adults were found in the caged or in the uncaged release area.

As indicated earlier by laboratory data, very few eggs hatched if the environment reached 35° C and RH dropped to 50% (Table 2).

Predator Studies.- It is quite possible that the failure of establishment of a field colony from a 2nd release of 50 adults in the summer of 1971 at the Johnson site was due to the presence of the carabid predator, Lebia viridis Say. Many Altica eggs and larvae were noted in the cage shortly after release, but the presence of this predator was unknown at that time. By the time individuals of L. viridis were detected, nearly all of the A. carduorum larvae had disappeared.

L. viridis closely resembles A. carduorum and can easily be mistaken for an Altica. Balsbaugh (1967) reported on several carabid-chrysomelid associations including ones involving L. viridis, in which possible mimicry occurs.

When it was recognized that this predator was present, 12 specimens were collected in one day. It is assumed that none of the A. carduorum larvae avoided

capture, since no adults emerged from this cage.

Based on samples of the flora and fauna, background counts for radioactivity at the Johnson farm averaged 25 cpm during the predator study. Samples of 3rd instar larvae, collected from the plants labeled with P^{32} ranged in activity from 1753 - 23,700 cpm. Adults varied in activity from 3200 - 3900 cpm.

Two plants which had been injected with P^{32} on September 1, 1972, were sampled 7 days later with each plant having over 30,000 cpm. Samples from the pitfall traps were analyzed at the end of the experimental period (October 8) and 1 coccinellid, Coleomegilla maculata Timberlake contained 2600 cpm, and 5 specimens of Harpalus pennsylvanicus De Geer, a carabid, ranged in counts from a low of 114 cpm to a high of 2560 cpm. All of this activity may not have come from the labeled Altica, however, because the Carabidae are scavengers and could have very easily ingested labeled plant debris, (Kirk, personal communication) or else obtained radioactivity from feeding on aphids which were present on the thistle. Based on these data it would appear that the 2 most likely carabid predators are Lebia viridis and H. pennsylvanicus.

In predation studies conducted by Riordan and

Peschken (1970) eggs were labeled by feeding adult beetles on thistles which had been vacuum impregnated with P^{32} solution. The advantage of using this method was that beetles were allowed to feed until preselected levels of radioactivity had been reached.

In the experiment conducted at the Johnson farm, I was not interested in the exact amount of P^{32} each life stage incorporated. A sufficiently high amount was injected into the plant to enhance its incorporation into the larvae and adults of A. carduorum, and thus a high amount would be incorporated into the predators, which was accomplished.

Mycoplasma Study.- Suspected mycoplasma-like bodies were located in the phloem of leaf vein samples from Canada thistle plants that displayed typical yellows symptoms (Fig. 5 and 6). These bodies were observed in cells devoid of other recognizable organelles or vacuoles. Not all cells in a stele contained these suspected mycoplasma-like bodies.

Healthy Canada thistle tissue is shown in Figs. 7 and 8. No mycoplasma-like bodies were observed in sections of noninfected control plants. In the sieve elements, these bodies are dispersed throughout the entire cytoplasma of the cell. The relative

Fig. 5.- A stand of Canada thistle, Cirsium arvense showing the symptoms of suspected yellows disease.

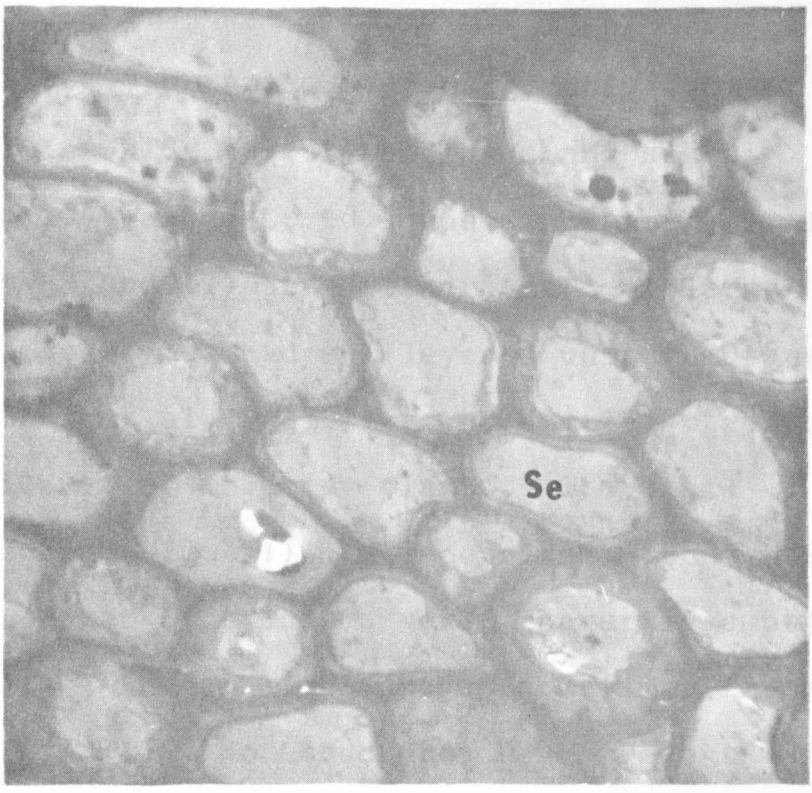
Fig. 6.- Close-up view of one Canada thistle plant showing the symptoms of suspected yellows disease.

5

6

Fig. 7.- Noninfected phloem tissue of Canada thistle. Sieve elements (Se). (Approx. X3200)

Fig. 8.- Noninfected phloem tissue of Canada thistle showing one sieve element (Se). Parenchyma (Pc). (Approx. X6100).



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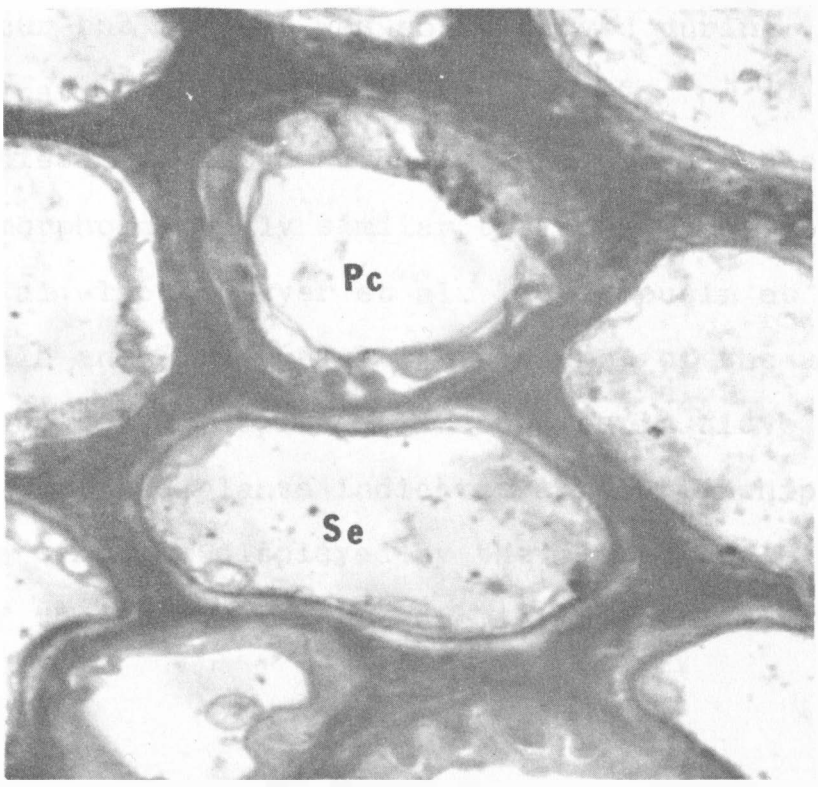

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abundance of these organisms varied greatly from cell to cell. A wide variety of forms and sizes (Figs. 9 and 10) was observed. The single spherical bodies (Figs. 9 and 11) were either small or large in size. These bodies had a 2 layered unit membrane (Fig. 10). Doi et al., (1967) found similar structures in phloem tissues of mulberry plants. The small spherical bodies (Fig. 11) had recognizable contents whereas the larger size bodies were apparently empty (Fig. 9). Also, the smaller spheres sometimes formed filaments (Fig. 11). No structures were present which would suggest budding or binary fission occurring in the spherical bodies. This is not to say that it does not occur but that it was not observed during this preliminary study.

The bodies asscciated with diseased Canada thistle plants are morphologically similar to known mycoplasmas (Hampton et al. 1969, Bowyer et al. 1969, Cousin et al. 1971, and Dale and Kim 1969). The presence of the above described suspected mycoplasma-like bodies in sieve elements of diseased plants indicates a relationship with disease symptoms displayed by the plant (the yellowing of the leaves), but there is no conclusive proof.

Fig. 9.- Suspected mycoplasma-like structures (Y) in Canada thistle leaf tissue. Cell wall (cw). (Approx. X10980).

Fig. 10.- Suspected mycoplasma-like bodies showing filamentous growth structures (Fs), and the 2 layered membrane (L). (Approx. X40,000).

9

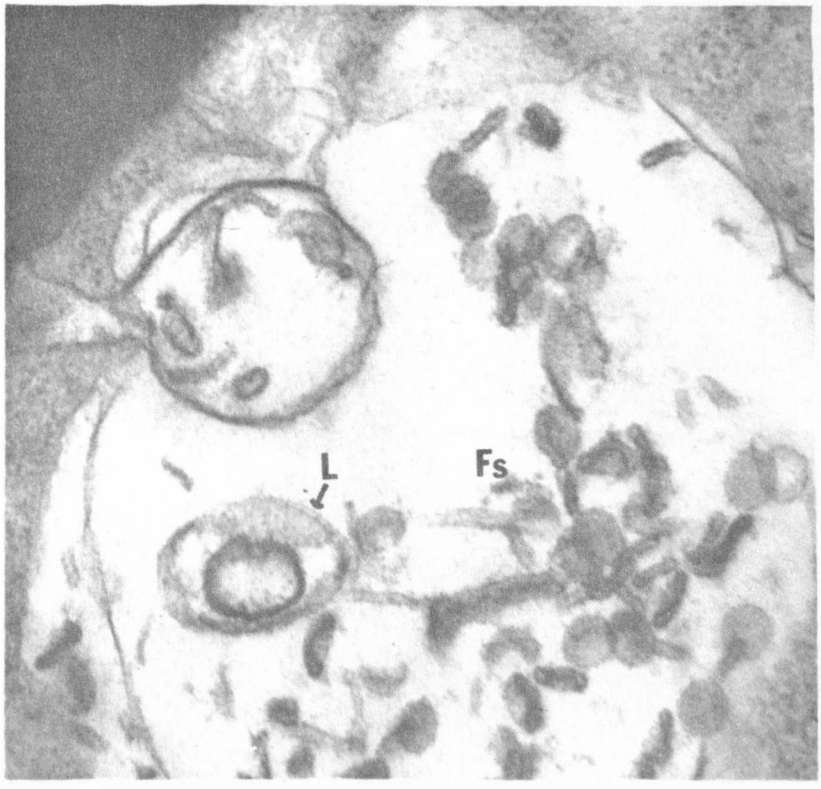

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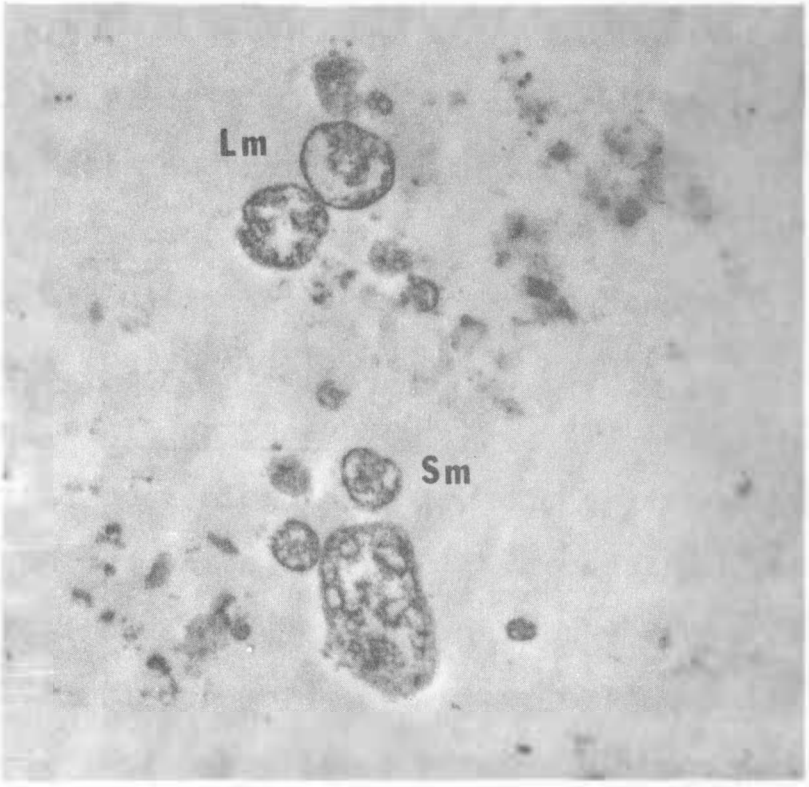
Fig. 11.- Individual suspected mycoplasma-like bodies showing the large (Lm), and the small (Sm) size spherical bodies. (Approx. X10,980).



11

Although most yellows disease-causing organisms affect many important food, forage, and horticulture plants, they also affect plants which are considered weeds. Thus, although weeds may play a role in the origin of yellows disease of crop plants, and serve as significant hosts in their perpetuation, it is hoped that through modern technology the utilization of these MLO's may ultimately serve in the control of noxious weeds (Duffus 1971).

Morphology.- Altica carduorum (Fig. 12) is a beetle having the posterior femur greatly enlarged and adapted for jumping, hence the name flea beetle. The head is visible from above, and the antennae are filiform. The tarsi appear 4-segmented, but actually are 5-segmented. These beetles are oval in shape and range in size from 3-5 mm. They are blue in color and the female is larger than the male. There is a complete absence of pubescence in this species.

The Head.- The hypognathous head* (Fig. 13) is visible from above and directed ventrad. The head

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*Explanation of abbreviations are given on pp. 65-66.

EXPLANATION OF TERMS

Abd	Abdomen
Acr	Acrotergite
Aed	Aedeagus
Ala	Alacristae
Anc	Antennal callus
Anf	Antennifer
Anp	Anterior notal process
Ans	Antecostal suture
Ant	Antenna
Apl	Apodemal lobes
Apt	Aperature
Apx	Apex
Atp	Anterior tentorial pits
Ats	Antennal sclerite
Axi	Axillary cord
Ax 1	
Ax 2	Axillaries
Ax 3	
Bas	Basalare
Bst	Basisternum
C	Costa
Car	Cardo
Cly	Clypeus
Cod	Condyle
Con	Convergent furrow
Cox	Coxa
Cpd	Compound eye
Den	Denticle
Dis	Discriminal line
Dpl	Dorsal plate
Dtp	Dorsal tentorial pits
Ely	Elytra
Epc	Epicranial suture
Epm	Epimeron
Eps	Episternum
Ept	Epistomal suture
Fem	Femur
For	Foramen magnum
Frc	Frontal callus
Frn	Frons
Frs	Frontal suture
Gal	Galea
Gen	Gena
Glo	Glossa
Gua	Gula
Gul	Gula suture
Hea	Head
Hup	Humeral plate
Kat	Katepisternum
Lab	Labium
Lac	Lacinia
Lap	Labial palpus
Lar	Labrum
Man	Mandible
Map	Maxillary palpus
Mef	Median foramen
Mem	Membraneous area
Men	Median plate
Mep	Mentum-prementum
Met	Median tongue
Mno	Mesonotum
Mst	Mesosternellum
Mus	Muscle disk
Obl	Oblique suture
Occ	Occiput
Ocs	Occipital suture
Ocv	Occipto-cervical condyles
Nec	Neck sclerites
Not	Notch
Paf	Palpifer
Pal	Palpiger
Par	Paraglossa
Pbs	Probasisternite
Ped	Pedicle
Pen	Penis
Phr	Phragma
Pit	Pits
Pls	Pleural wing process
Pnp	Posterior notal process

Pre Prementum
Prc Procoxa
Pro Pronotum
Prs Prescutum
Prt Prosternellum
Pth Prostheca
Pti Prebasal transverse impression
Ptn Postnotum
Ptp Posterior notal process
Ptx Prothorax
Pul Pulvillus
Pwp Pleural wing process

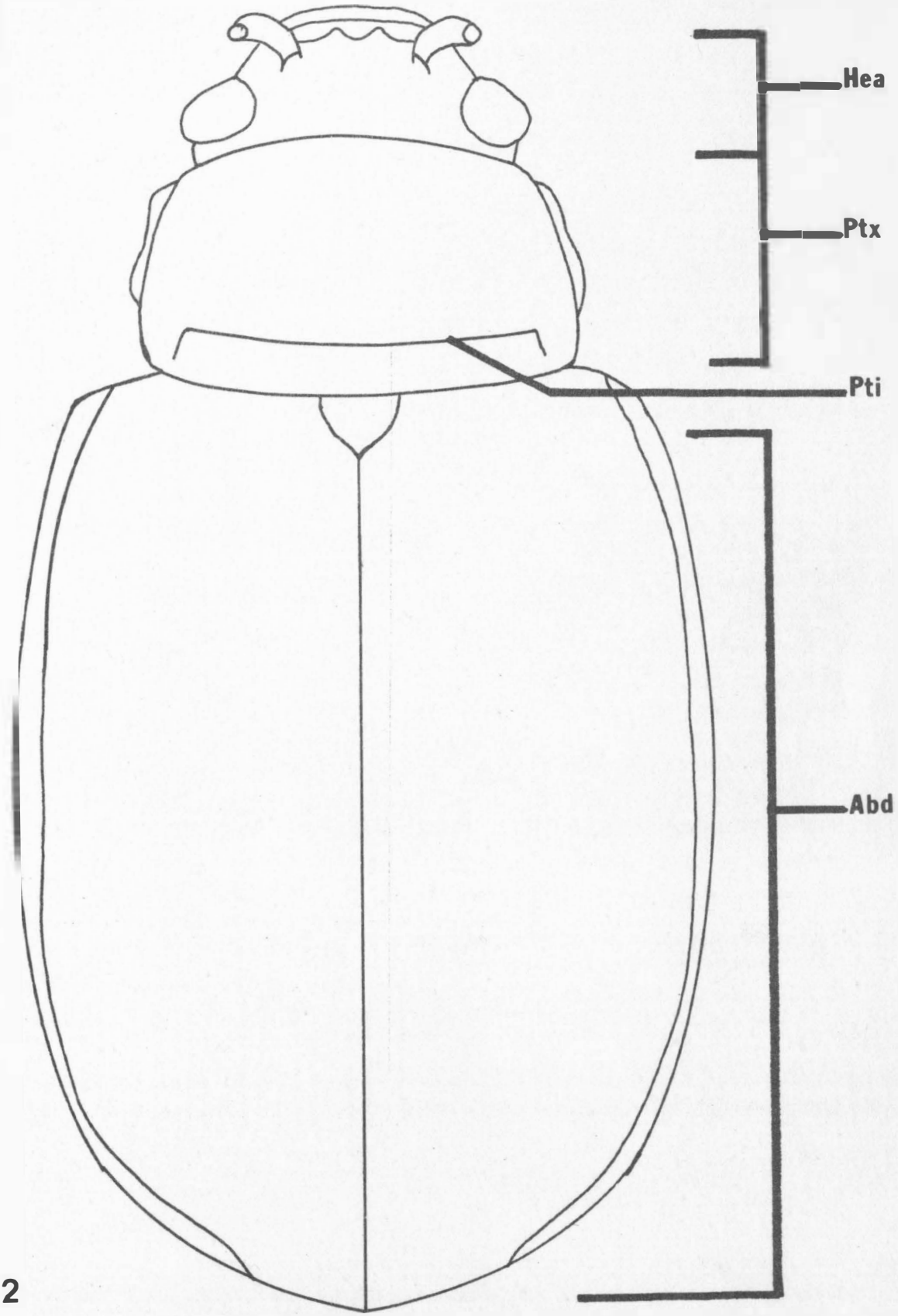
Rid Ridge
Rns Reverse notal suture

Scp Scape
Scm Scutum
Scs Scuto-scutellalar suture
Sct Scutellum
Seg 4th tarsal segment
Smt Submentum
Spi Spiracle
Spm Spine
Stn Sternellum
Stp Stipes
Sts Sternal suture
Sub Subalare
Sus Sub-antennal suture

Tac Tarsal claw
Tar Tarsus
Teg Tegmen
Tib Tibia
Trc Trochantor
Tro Trochantin

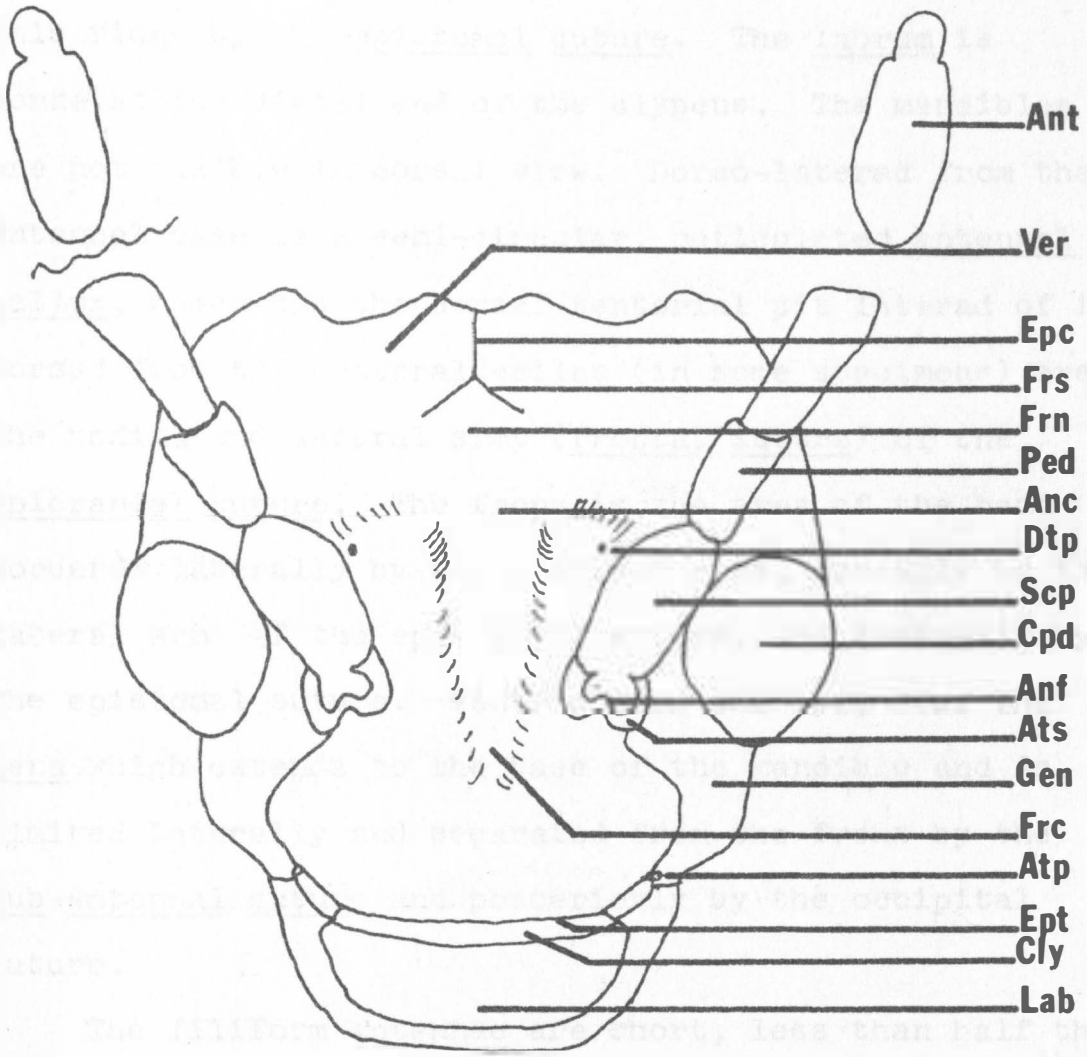
Ver Vertex

Fig. 12.- A. carduorum, dorsal view.



1 2

Fig. 13.- A. carduorum head, dorsal view.


Ant
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Epc
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Cly
Lab

13

capsule is dark blue. The median area has a frontal callus or ridge running anteriorly in the area between the antennae sockets and expanding laterally above the labrum. The clypeus is separated from the base of this ridge by the epistomal suture. The labrum is borne at the distal end of the clypeus. The mandibles are not visible in dorsal view. Dorso-laterad from the antennal base is a semi-circular, reticulated antennal callus, which has the dorsal tentorial pit laterad of it. Dorsad from the antennal callus (in some specimens) are the medial and lateral arms (frontal suture) of the epicranial suture. The frons is the area of the head bordered laterally by the compound eyes, dorsally by the lateral arms of the epicranial suture, and ventrally by the epistomal suture. Ventrad from the eye, lies the gena which extends to the base of the mandible and is limited laterally and separated from the frons by the sub-antennal suture and posteriorly by the occipital suture.

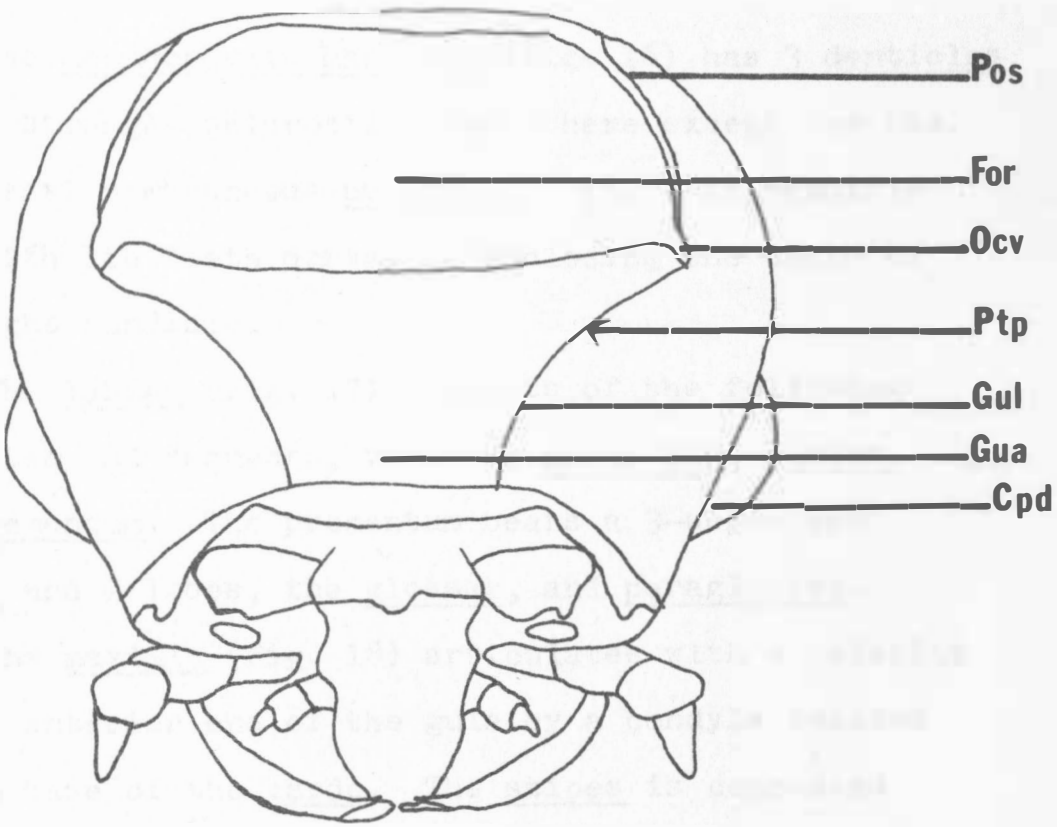
The filiform antennae are short, less than half the length of the body and arise laterad to the compound eyes. The scape and articles 3-11 are of equal length. The pedicle is about half the length of the other articles.

The anterior tentorial pits lie between the genae and the sub-genae in the fronto-genal - sub-genal suture. No tormae are noticed except in the ventral view of the labrum. No neck sclerites are visible dorsally. Presumably, the head articulates on the occipito-cervical condyles.

The posterior section of the head (Fig. 14) consists of the foramen magnum, postocciput, occiput, gena and vertex. No occipital suture is visible. The compound eyes are located on the meso-lateral margins. The foramen magnum is bounded laterally and dorsally by the post-occipital suture which laterally separates the post-occiput from the gena. The posterior region of the head is comprised of the fused vertex and occiput. Located within the foramen magnum at the anterior end of the gula suture can be seen the occipito-cervical condyles, which permit the movement of the head. The deep posterior tentorial pits are located (Fig. 14 arrow) in the dorsal 1/3 of the gula suture.

The labrum is attached to the distal end of the clypeus. The dorsal surface is completely sclerotized and has several small setae. The ventral surface (Fig. 15), which forms the epiphraynx, is less distinctly sclerotized; however, there is a large,

Fig. 14.- A. carduorum head, ventral view.



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recurved, heavily sclerotized spine-like structure on the baso-mesal margin. The labrum is slightly notched and has 2 small pits on either side of the median line.

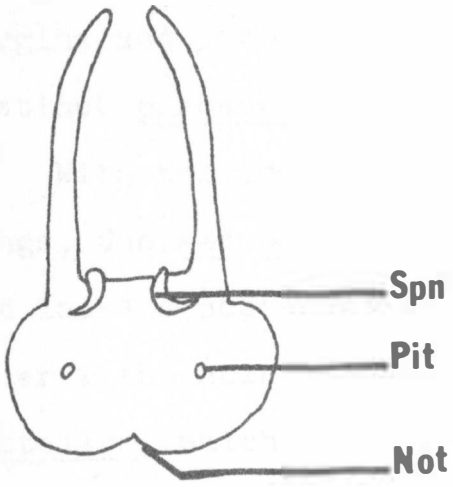
The monocondylic mandible (Fig. 16) has 3 denticles and is strongly sclerotized everywhere except for the base-mesal membraneous prostheca. The left mandible lies with its teeth dorsad or enclosing the teeth of the right mandible.

The labium (Fig. 17) consists of the following sclerites and segments, viz. the submentum, mentum, and prementum. The prementum bears a 3-segmented palpus and 2 lobes, the glossae, and paraglossae.

The maxilla (Fig. 18) articulates with a sclerite at the anterior end of the gula by a condyle located at the base of the cardo. The stipes is connected distally with the cardo and bears 3 freely articulating appendages which are directed mesal, viz. the palpus, lacinia, and galea, respectively, from lateral to mesal insertions. The stipes is separated from the head proper by a membrane. The distal tip of the galea bears 6 long, apically recurved hairlike projections.

The Thorax.- The pronotum (Fig. 12) is covered by a large somewhat rectangular tergite with explanate

Figs. 15-18.- A. carduorum. 15, Labrum, ventra
view; 16, right mandible, ventral view; 17, right
labium, ventral view; 18, right maxilla, ventral vie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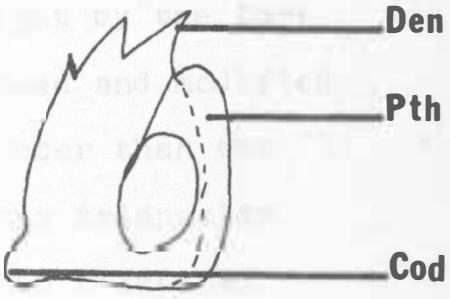
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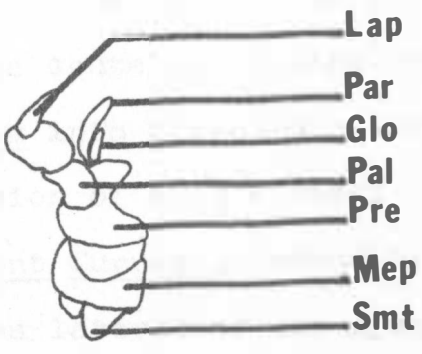
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Glo
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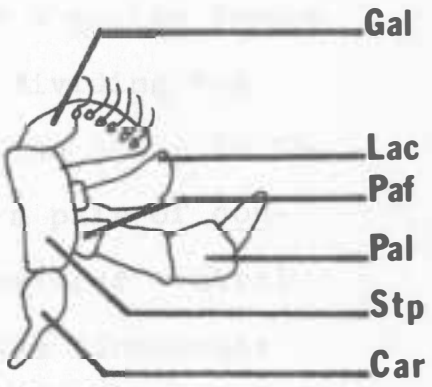
Pre
Mep
Smt

17


Gal
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Car

18

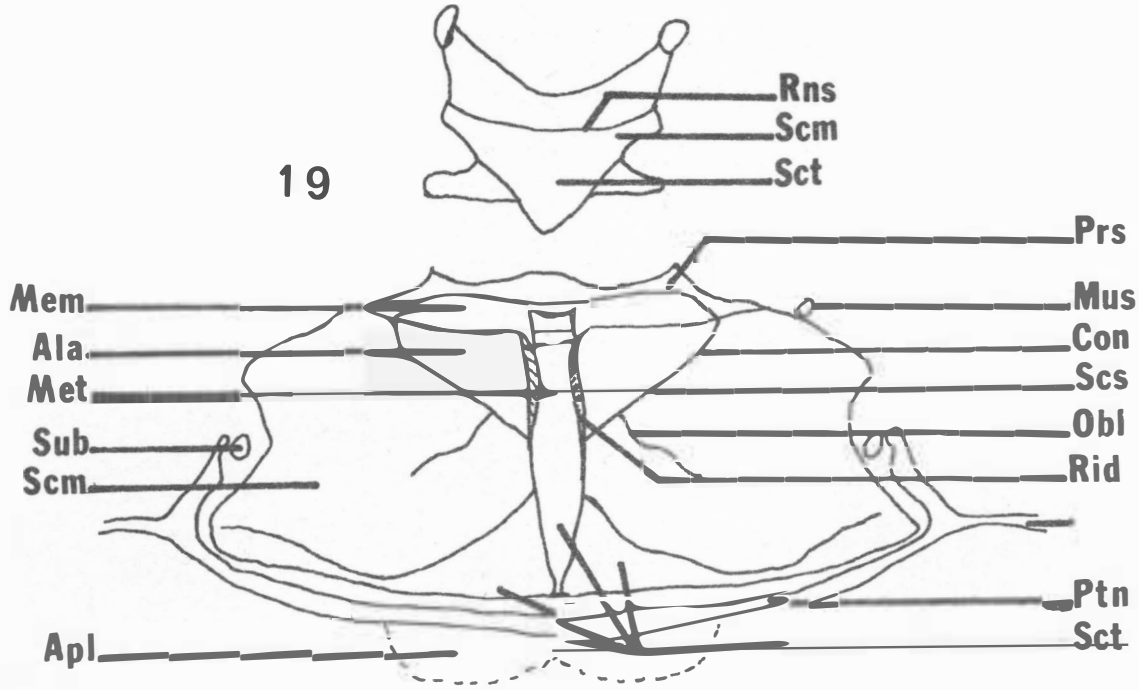
margins and altogether is as wide as the elytra. A distinct prebasal transverse impression is present.

With the loss of function for flight by the fore wings, the mesonotum (Fig. 19) is reduced and modified and lacks a postnotal area. It is shorter than the other 2 thoracic segments, and the large triangular scutellum, which divides the scutum into 2 lateral halves overlaps the metanotum. The acrotergite (Figs. 21, 22) is a narrow sclerite just preceding the base of the scutellum, set off by the antecostal suture. A reverse notal suture (Fig. 19) is present in the posterior part of the alinotum near the base of the elevated scutellum. The scutellar region is divided into a median elevated shield and 2 lateral areas (Fig. 20).

The metathorax of Coleoptera has 2 special modifications. One is the forward extension of a median tongue of the scutellum toward the prescutum, dividing the scutum into 2 separate lateral lobes. The other is the division of each lateral lobe again by a pair of convergent furrows, formed by special transverse ventral ridges latered of the apex of the V-ridge (Snodgrass 1909).

Figs. 19, 20.- A. carduorum. 19, Mesothorax, dorsal view; 20, metathorax dorsal view.

19


Rns
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Scs
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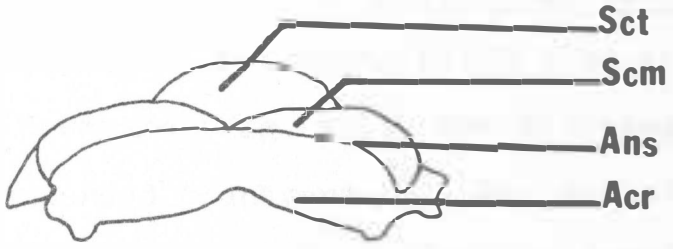
20

Figs. 21, 22.- A. carduorum. 21, Mesothorax, anterior view; 22, metathorax, anterior view.



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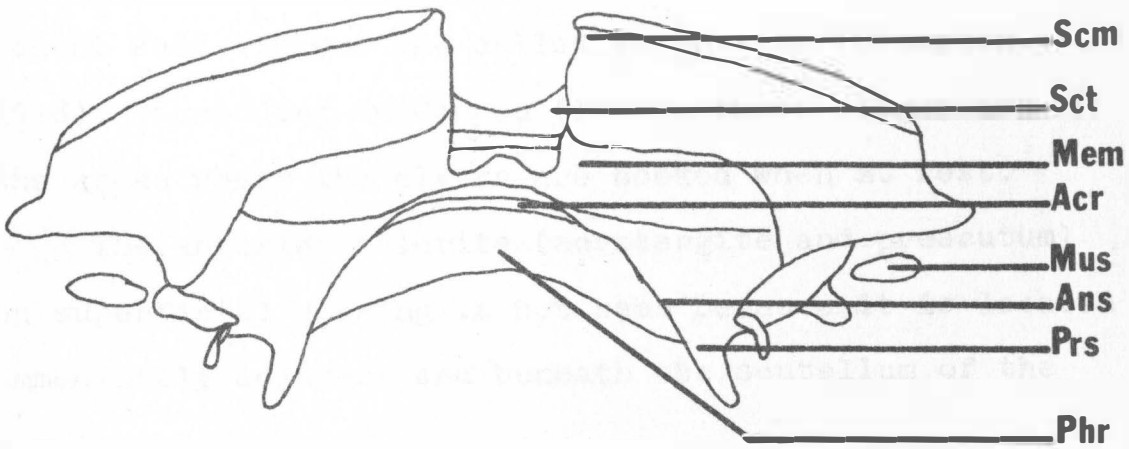


2 2

In Altica the median part of the prescutum is narrow and arched forward. The antecostal suture separates it from the acrotergite which lies anteriorly and is flexed downward (Fig. 20). The membraneous area behind it is extended transversely. The scutellum has a long median tongue extending the entire length of the metathorax, separating the scutum into lateral halves. The 2 lateral areas are partially divided into anterior and posterior parts by the intrascutal suture (oblique suture). In front of the convergent furrows the anterior scutal subdivisions are called alacristae (Crampton 1918). According to Campau (1940), these ridges are the areas where the elytra are hooked when at rest.

The anterior sclerite (acrotergite and prescutum) on superficial viewing is not seen because it is located immediately adjacent and beneath the scutellum of the mesotergum.

The ental portions of the postnotum bear vertical intersegmental plate-like apodemal lobes (=phragma) (Fig. 20). These are medially emarginate to allow for the passage of the dorsal blood vessel (Snodgrass 1935). The lateral extensions fuse with the sclerite in front of them so that the axillary cord appears to be attached to the margins of the scutum (Fig. 20).

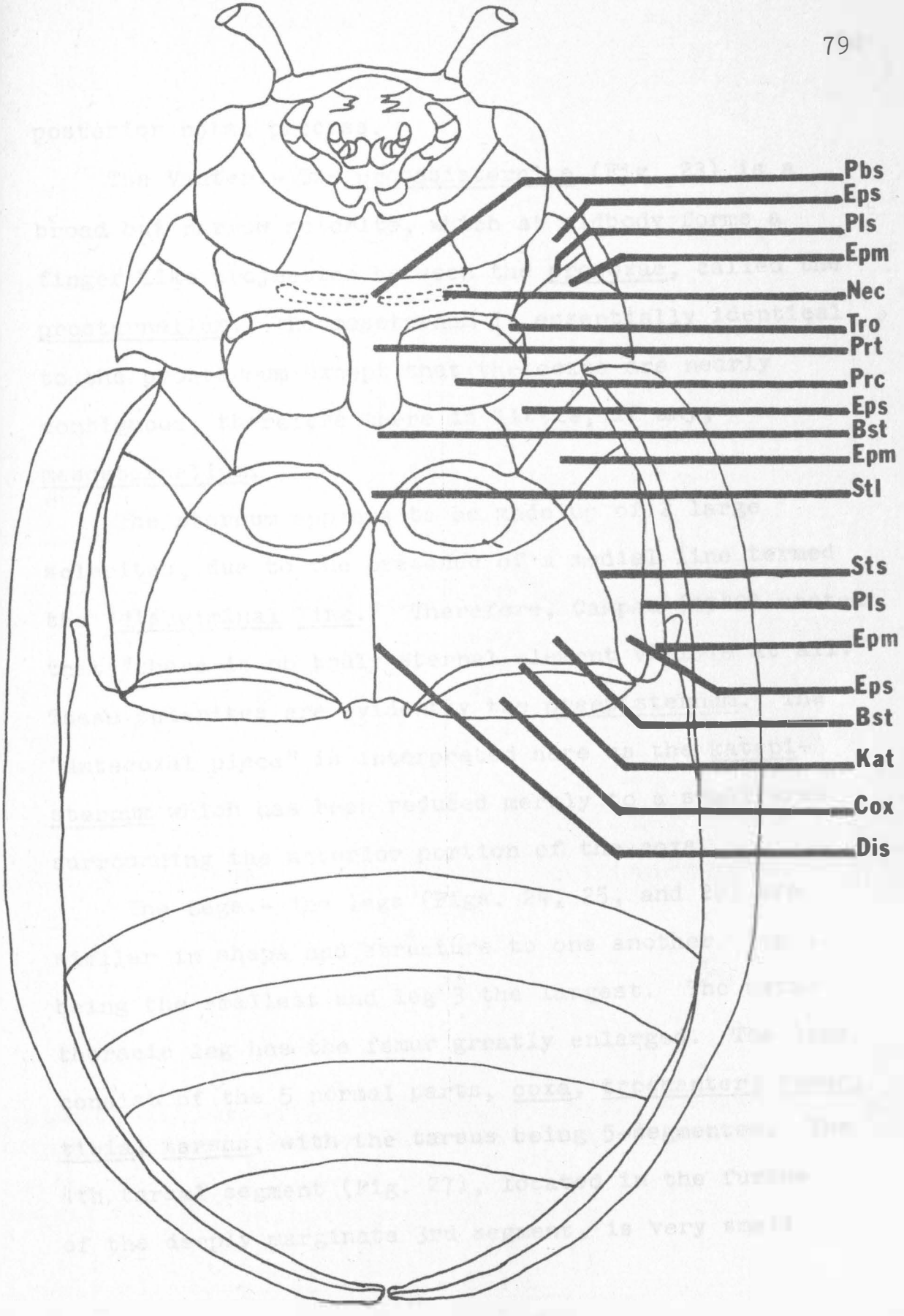
The Pleura.- The pleura (Fig. 23) of the pro- and mesothorax resemble each other, but are vertical and oblique, respectively. The prothoracic pleurites seem fused with each other and with the tergum and sternum, but are not reduced in size. The pleural suture separates the pleurites of the pro- and mesothorax into episternum and epimeron, which are separated from the sternum by a ridge, the sternal suture. The pleurites of the prothorax are joined to the protergite.

The pleura of the metathoracic segment do not differ fundamentally from the other pleura but have only secondary differences. These are nearly horizontal. Arising from the anterior end of the pleurites is the pleural wing process. The pleural suture has its anterior end bent slightly downward, then straightening, ending abruptly at the metacoxa. No further sutures divide the pleurites.

The trochantin can be located only on the pro- and mesocoxae.

The basalare, the anterior epipleurite of the metathoracic segment, is located at the anterior tip of the episternum and appears to be somewhat fused with it. The subalare, (Fig. 30) the posterior epipleurite, is free and lies laterad and somewhat basad to the

Fig. 23.- A. carduorum. ventral view.


Pbs
Eps
Pls
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Pls
Epm
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Bst
Kat
Cox
Dis

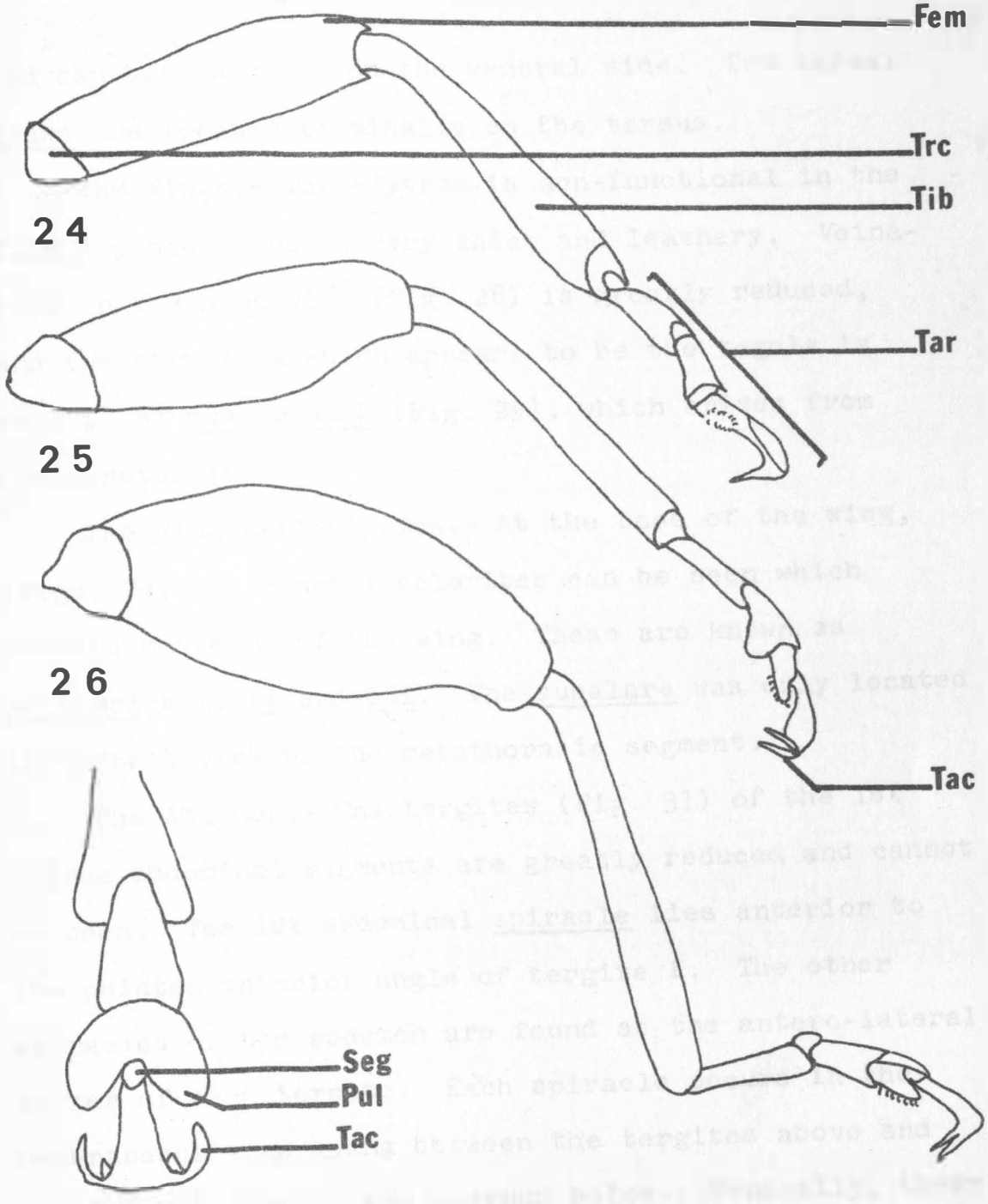
posterior notal process.

The Venter.- The probasisternite (Fig. 23) is a broad but narrow sclerite, which at midbody forms a finger-like projection between the procoxae, called the prosternellum. The mesoternum is essentially identical to the prosternum except that the coxae are nearly contiguous, therefore there is little, if any, mesosternellum.

The sternum appears to be made up of 2 large sclerites, due to the presence of a medial line termed the "discriminal line." Therefore, Campau (1940) stated that "there is no truly sternal element visible at all." These sclerites are evidently the preepisternum. The "antecoxal piece" is interpreted here as the katepisternum which has been reduced merely to a small area surrounding the anterior portion of the coxa.

The Legs.- The legs (Figs. 24, 25, and 26) are similar in shape and structure to one another, leg 1 being the smallest and leg 3 the largest. The metathoracic leg has the femur greatly enlarged. The legs consist of the 5 normal parts, coxa, trochanter, femur, tibia, tarsus, with the tarsus being 5-segmented. The 4th tarsal segment (Fig. 27), located in the furrow of the deeply marginate 3rd segment, is very small

Figs. 24-27.- A. carduorum. 24, Prothoracic leg; 25, mesothoracic leg; 26, metathoracic leg; 27, tarsi I-V, ventral view.



24

25

26

27

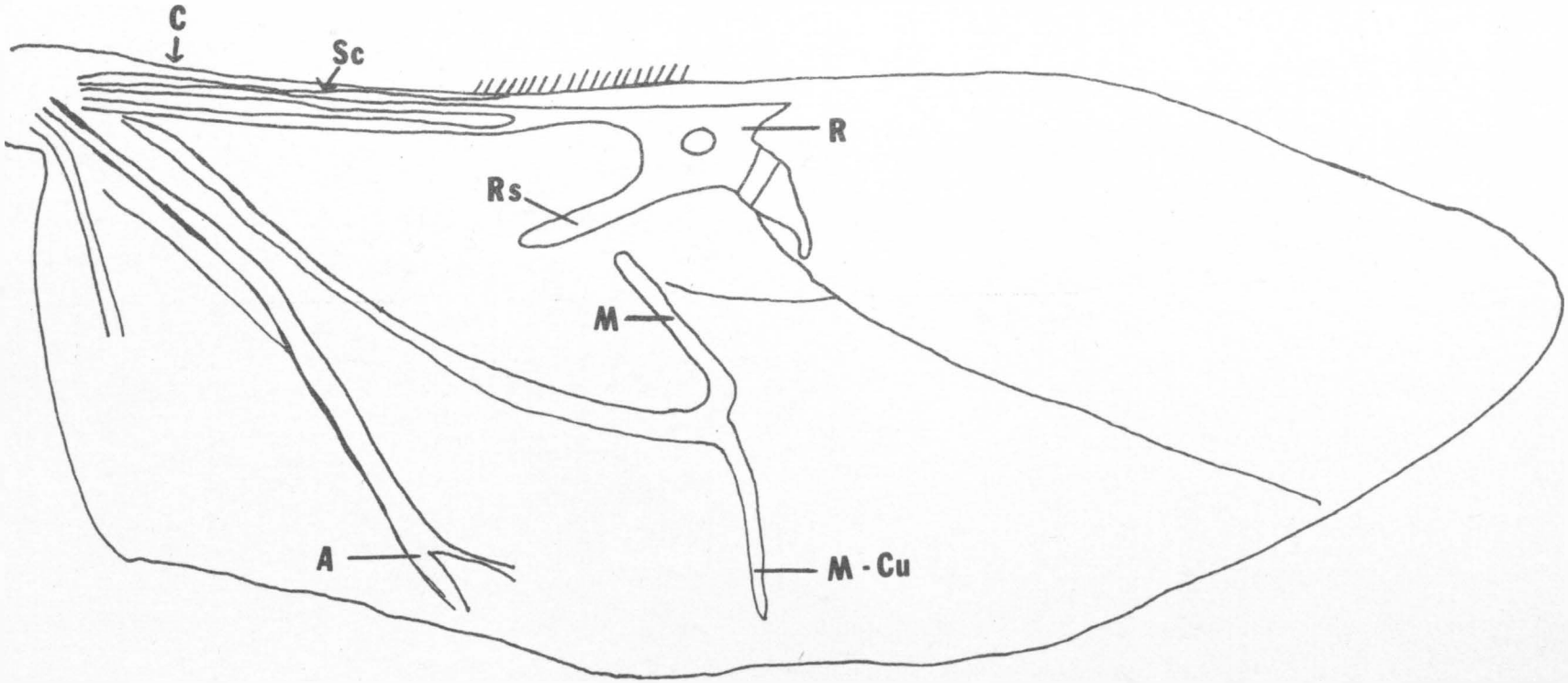
and can best be seen on the ventral side. Two tarsal claws are located terminally on the tarsus.

The Wings.- The elytron is non-functional in the flight process and is very thick and leathery. Veination in the hind wing (Fig. 28) is greatly reduced, and the structure which appears to be the tegula is really the muscle disk (Fig. 29), which arises from the acrotergite.

The Wing Articulation.- At the base of the wing, (Figs. 29, 30) 3 small sclerites can be seen which permits movement of the wing. These are known as axillaries I, II and III. The subalare was only located in ventral view of the metathoracic segment.

The Abdomen.- The tergites (Fig. 31) of the 1st 2 true abdominal segments are greatly reduced and cannot be seen. The 1st abdominal spiracle lies anterior to the pointed anterior angle of tergite I. The other spiracles of the abdomen are found at the antero-lateral corner of each tergite. Each spiracle occurs in the membraneous area lying between the tergites above and the lateral edge of the sternum below. Ventrally, there are 5 visible abdominal sternites.

Fig. 28.- A. carduorum, right metathoracic wing, dorsal view.



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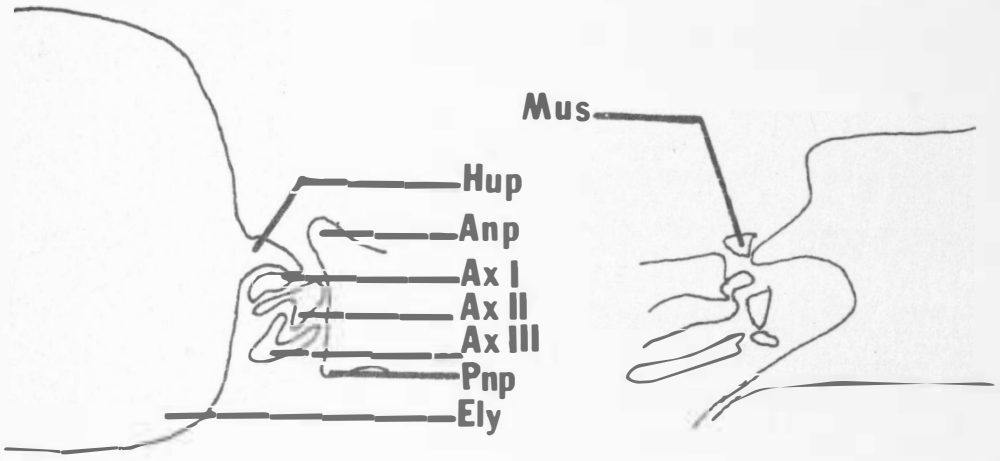
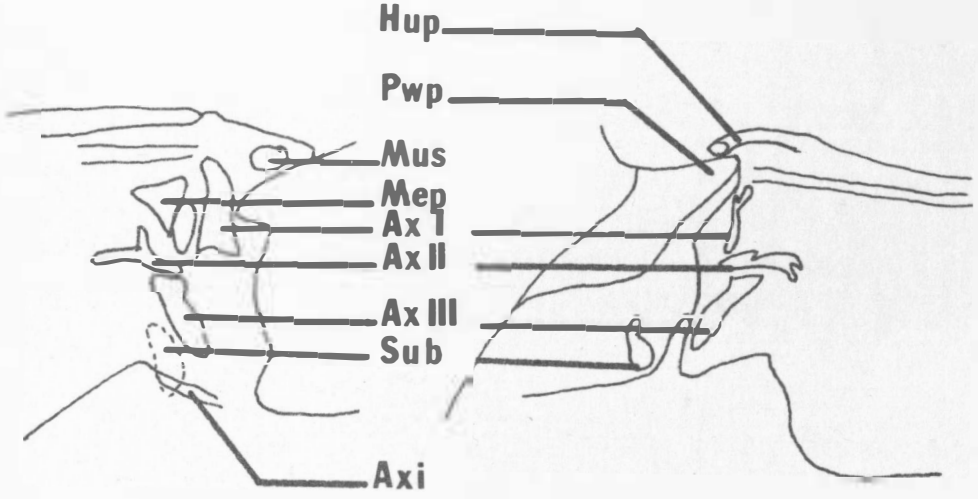
Figs. 29, 30.- A. carduorum. 29, Mesothoracic wing articulations, dorsal view; 30, metathoracic wing articulations, ventral view.


Mus
Hup
Anp
Ax I
Ax II
Ax III
Pnp
Ely

29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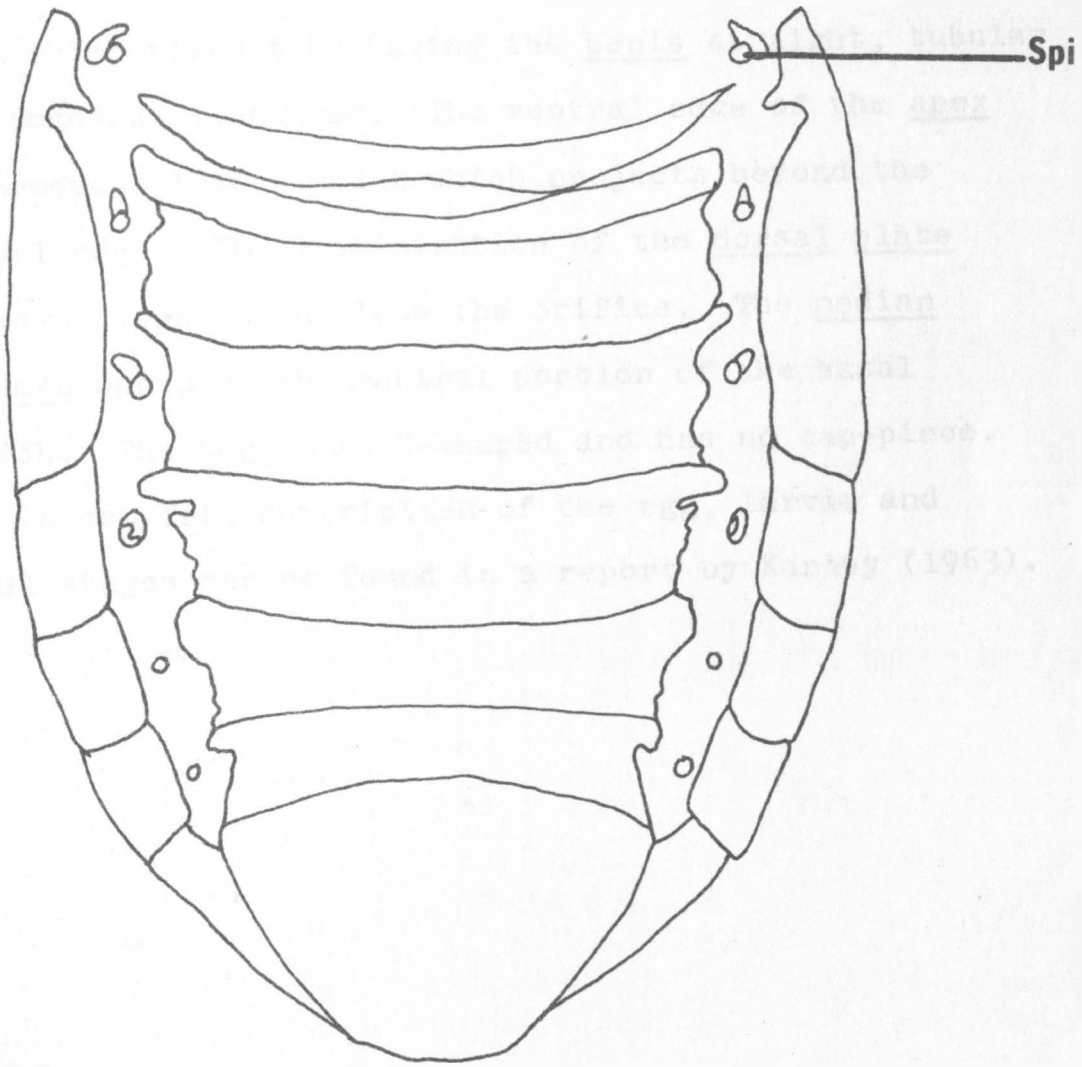
30

Fig. 31.- A. carduorum, abdomen, dorsal view.

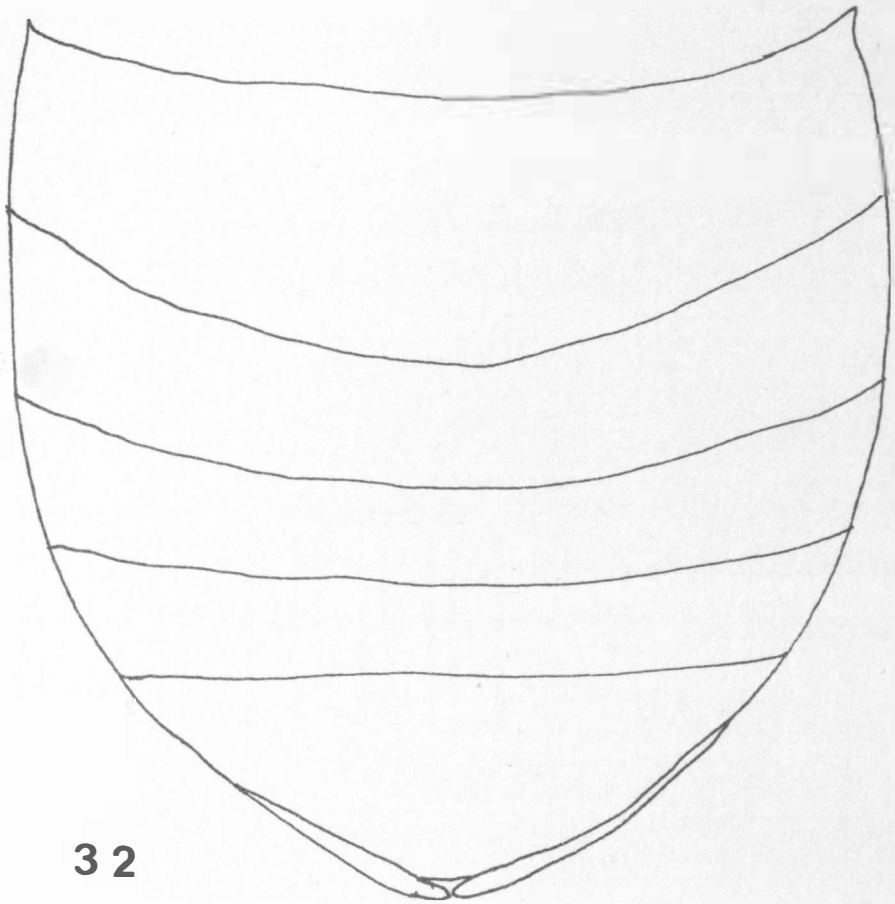


31

Male Genitalia.- The males may be separated from the females by the structure of abdominal segment VII. In the females the posterior margin is entire (Fig. 32). However, in the male this same edge is emarginate medially, which allows for the extension and retraction of the aedeagus (Fig. 33). The aedeagus (Figs. 34, 35) is a sclerotized tube having the penis straight, tubular and somewhat flattened. The ventral edge of the apex is produced into a point which projects beyond the dorsal edge. The chitinization of the dorsal plate forms 3 flaps which close the orifice. The median foramen occupies the ventral portion of the basal fourth. The tegmen is Y-shaped and has no cap-piece.

A complete description of the egg, larvae and pupal stages can be found in a report by Karney (1963).

Figs. 32, 33.- A. carduorum. 32, ♀, abdomen, ventral view; 33, ♂, abdomen, ventral view.



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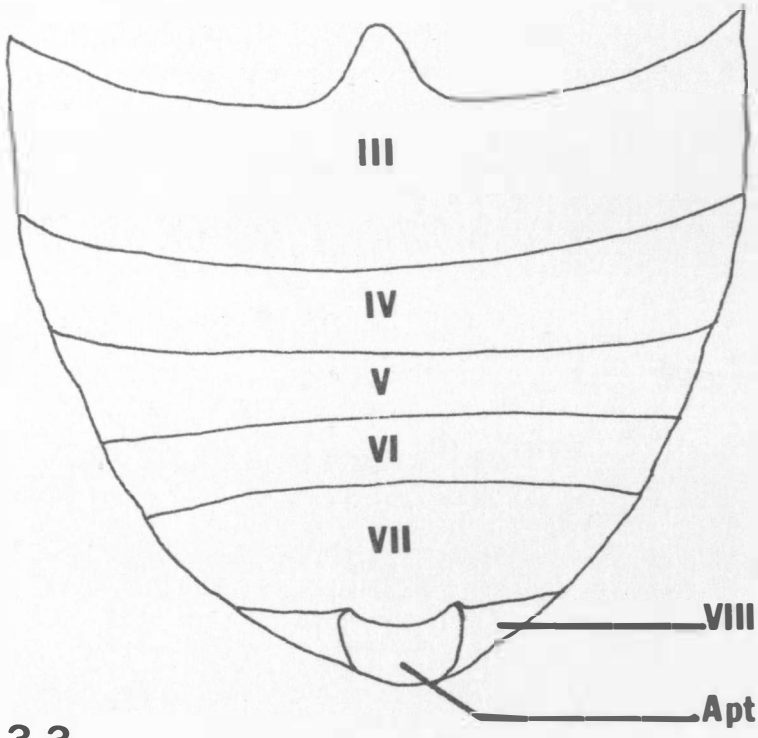
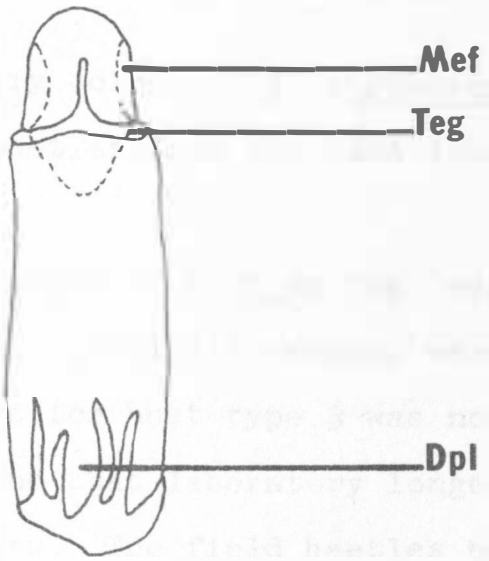


3 3

Figs. 34, 35.- A. carduorum. 34, Male genitalia, dorsal view; 35, male genitalia, ventral view.



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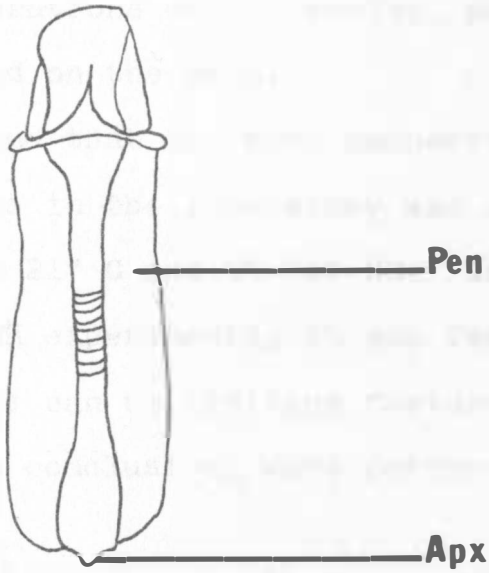

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SUMMARY AND CONCLUSIONS

A laboratory colony of A. carduorum was established from 50 adult beetles from the USDA laboratory, Albany, California.

Four host-types of Cirsium spp. were screened and although no statistical differences were shown, a distinct preference for host-type 3 was noted.

It was found that laboratory longevity of adults averaged 100 days. The field beetles have a much shorter adult life, but 1 beetle lived 100 days in the field. If 2 adult generations would overlap, much more control could be exerted on the weed.

It was found that the best temperature at which to rear the beetles in the laboratory was a day temperature of 30° C, night 21° C and 50-70% RH. In performing the temperature - RH experiments, it was found that both these parameters can be limiting factors to life of the beetles. These conclusions were corroborated by field data.

Although the findings are preliminary, it appears that some life stages of A. carduorum may be reared on artificial diet. If rearing techniques were perfected many hours of manual labor could be cut from the regular

maintenance of a plant-fed laboratory colony.

Mycoplasma-like bodies were found in C. arvense displaying the classic symptoms of yellows disease. Although Canada thistle may play a role in the origin of this disease and serve as a host, it is hoped that modern technology ultimately can utilize the mycoplasma-like organisms in the control of weeds.

The complete external morphology of the adult beetle is described.

Following the success of the release at the Johnson farm, other field releases were made. This was the only place in the United States where overwintering adults have laid viable eggs. Only at one other place, Lacombe, Alberta, Canada, has this success been duplicated.

The major factors limiting the establishment of field colonies are predators viz. L. viridis, and H. pennsylvanicus; very high temperatures; low RH; and combinations of the above.

If A. carduorum is to be used for biological control of Canada thistle, several problems must be overcome, viz. the prevention of a fall decline in egg laying and hatching in the laboratory colony. Such

declines in fecundity probably result because of feeding on fall field thistle. Possibly, the host plant stimulates a response in the beetle to prepare for winter and reduced fecundity is a result of this.

A laboratory colony must be maintained to determine further effects of temperature and RH on fecundity, and to produce excess adults for releases at additional sites within the state.

The greatest limiting aspect in this research was the inability to mass produce adult beetles when they were needed.

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